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NEWCASTLE DISEASE OF FOWLS.*

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NEWCASTLE disease was first observed in 1926 on a poultry farm near Newcastle-on-Tyne. During the following six months the disease was diagnosed by this laboratory in eleven counties; it is reasonable to assume that many other outbreaks occurred, as some are known to have been diagnosed by other laboratories and some were probably not reported.

In all the reported outbreaks in this country the mortality was 98-100 per cent. and it was probably because of the absence of survivors that widespread dissemination of the disease did not occur and that it quickly died out.

No further outbreaks were diagnosed in Britain until the autumn of 1933 when the disease appeared on a large poultry farm. Six thousand fowls either died or were destroyed, but owing to the rigorous measures of isolation and disinfection carried out no secondary centres of the infection occurred.

Picard (1928) reported that in 1926 a new disease of fowls was observed in the neighbourhood of Batavia, Dutch East Indies. It spread rapidly and within a year the whole Dutch Indian Archipelago was infected. The mortality was nearly 100 per cent. and vast numbers of fowls died. It was clear from Picard's description of the disease that it was very similar to the infection present in England.

Rodier (1928) reported from the Philippine Islands what he believed to be a new disease of fowls. It first appeared in Manila and then spread rapidly over a wide area of country, causing the

*The term " Newcastle disease " was given provisionally when it was first recognised to be a separate entity ; it is obviously unsuitable, yet it would appear advisable to retain it until such time as a more appropriate one is coined. The disease should not be so named as to create confusion with an existing disease ; therefore, the only suggested alternatives " pseudo-plague " or " avian pseudo-pest " should be avoided.

death within a few months of at least 50,000 fowls. His description of the disease strongly suggested that it was similar to the infections present in England and in the Dutch Indian Archipelago. Affected fowls died in from one to seven days from the onset of symptoms. He pointed out that although the Philippine disease bore some resemblance to fowl plague he believed it to be a separate entity.

Farinas (1930) stated that the Philippine disease had been observed in fowls, ducks, turkeys, guinea-fowls, pigeons, parrots and wild birds. The period of incubation following artificial infection was from four to five days and the duration of visible illness from two to four days. The average mortality from natural infection was about 90 per cent.

In the annual report for 1927-28 of the Director (Dr. J. T. Edwards), Imperial Bacteriological Laboratory, Muktesar, India, reference was made to a highly virulent disease of fowls that had been observed in the Kumaon Hills.

This disease was studied subsequently by Cooper (1931), who described it under the title of " Ranikhet Disease." He stated that it had been reported from many widely separated areas and that in some localities crows were said to have died in large numbers.

He tested the susceptibility of 113 Indian country-bred fowls to artificial infection with the virus with the following results :—

Reacted and died—96 fowls or 85·0 per cent.
Reacted and recovered—11 fowls or 9·7 per cent.
No appreciable reaction—6 fowls or 5·3 per cent.

The Indian Official Delegate to the World's Poultry Congress, London (1930), stated that the disease had killed off millions of fowls in India.

In 1929 Newcastle disease was reported from Ceylon, where it spread rapidly and caused heavy losses.

The symptoms exhibited by affected fowls, as described by Crawford, were similar to those observed in England, Dutch East Indies, the Philippine Islands and India. The mortality was usually about 100 per cent. The disease was observed in fowls, turkeys, guinea-fowls and crows.

In view of the prevalence of the disease in Southern India the Government of Ceylon in 1931 prohibited the importation of poultry from that country.

Konno, Ochi and Hashimoto (1929) investigated an infection of fowls known as the " Korean poultry disease " and they concluded that it was identical with Newcastle disease and with the disease present in the Dutch East Indies. It is caused by a filterable virus and is almost invariably fatal. The most characteristic symptom is respiratory distress.

Nakamura, Oyama, Fukosho and Tomonga (1933) reported the same disease in Korea and in Japan.

A serious outbreak of Newcastle disease was reported in 1931 from Australia. A report states :—

" It is not known how the disease first originated in Victoria, but it is assumed that it has been imported from overseas, either by the introduction of poultry or cage birds, because the disease has never been previously known in Australia. The severity of the infection and the seriousness of the position can be realised when it is pointed out that the mortality is 100 per cent. of the affected birds.

" It is certain that only the stringent action taken by the Victorian Department in placing the whole State in quarantine, and closing up all auction rooms, had checked the spread of a disease which threatened to wipe out the entire poultry industry in Victoria and also in the adjoining States."

It is a noteworthy fact that the descriptions given by various authors of Newcastle disease as it was observed in England, Dutch East Indies, Philippine Islands, India, Ceylon, Korea and Japan have agreed as regards the causal agent, period of incubation, symptoms, lesions and infectivity.

The wide distribution of the disease, its high infectivity, and great virulence, render it a serious menace to all countries in which poultry farming is extensively practised.*

Cross-Immunity Tests with Strains of Newcastle Virus.

Owing to the great difficulty of immunising fowls on any considerable scale against Newcastle virus we arranged an interchange of strains with Picard in Java and Rodier in Manila, and each tested the other strains on fowls hyperimmunised against their own strain.

Cross-immunity tests were in this way carried out with the Newcastle virus and the Philippine virus, and with the Newcastle virus and the Java virus. The following tables show the results obtained in tests carried out at Manila and at Weybridge.

CROSS-IMMUNITY TESTS BETWEEN NEWCASTLE VIRUS AND THE PHILIPPINE VIRUS CARRIED OUT BY DR. E. A. RODIER AT MANILA, PHILIPPINE ISLANDS, AND PUBLISHED BY HIS PERMISSION.

Newcastle Virus. Received May, 1929.

Two tubes containing pieces of spleen in 50 per cent. glycerine saline.
Three tubes containing mouth exudate in 50 per cent. glycerine saline.

Test I.

Suspension of Newcastle spleen in 50 per cent. glycerine saline.

Fowl 344, 1 c.c. intramuscularly. Died 6th day. Typical disease ; good lesions.

Fowl 345, 1 c.c. intramuscularly. Died 10th day. Typical disease ; fair lesions.

*Experiments carried out at this laboratory have proved that Newcastle virus remains viable for at least six months in the bone marrow and muscle of carcases stored under trade chilling conditions.

Fowls immune to the Philippine Virus.

Fowl 9, 1 c.c. intramuscularly. Remained healthy.
Fowl 10, 1 c.c. intramuscularly. Remained healthy.
Fowl 19, 1 c.c. intramuscularly. Remained healthy.
Fowl 12, 1 c.c. intramuscularly. Remained healthy.

Test II.

Suspension of Newcastle mouth exudate (No. 1) in 50 per cent. glycerine saline.

This sample of virus was apparently dead, no symptoms appearing in the inoculated fowls.

Test III.

Suspension of Newcastle spleen (No. 2) and mouth exudate (Nos. 2 and 3) in 50 per cent. glycerine saline.

Fowl 348, 1 c.c. subcutaneously. Died 7th day. Typical disease ; good lesions.

Fowl 349, 1 c.c. subcutaneously. Sick and recovered after exhibiting typical symptoms.

Fowls immune to the Philippine Virus.

Fowl 9, 1 c.c. subcutaneously. Remained healthy.
Fowl 10, 1 c.c. subcutaneously. Remained healthy.
Fowl 11, 1 c.c. subcutaneously. Remained healthy.
Fowl 12, 1 c.c. subcutaneously. Remained healthy.
Fowl 13, 1 c.c. subcutaneously. Remained healthy.
Fowl 14, 1 c.c. subcutaneously. Remained healthy.
Fowl 15, 1 c.c. subcutaneously. Remained healthy.
Fowl 16, 1 c.c. subcutaneously. Remained healthy.

Test IV.

Mixed suspension of brain, kidney, liver and spleen of Fowl 344, dead of Newcastle disease.

Fowl 346, 1 c.c. subcutaneously. Died on 23rd day after exhibiting paralysis.

Fowl 347, 1 c.c. subcutaneously. Killed on 4th day for virus. Typical symptoms.

TEST IV.

CROSS-IMMUNITY TEST : NEWCASTLE VIRUS *VS.* FOWLS IMMUNIZED WITH PHILIPPINE VACCINE.

Fowl.	*Material.*	*Dose.*	*Route and Date.*	*Results.*
Immune Fowl No. 338	Suspension of spleen, brain, liver and kidney of fowls dead from New-Virus	0·5 c.c.	Subcutaneously 5.7.29	Remained normal up to 18.7.29.
Immune Fowl No. 341	Ditto	Ditto	Ditto	Ditto
Immune Fowl No. 342	Ditto	Ditto	Ditto	Ditto
Susceptible Fowl No. 354 of Test VI Control	Ditto	Ditto	Ditto	Became sick. Symptoms typical ; lesions good. Died 13.7.29.
Susceptible Fowl No. 355 of VI Control	Ditto	Ditto	Ditto	Became sick. Symptoms typical ; lesions good. Died 12.7.29.

TEST V.

NEUTRALISATION OF NEWCASTLE VIRUS BY PHILIPPINE IMMUNE SERUM.

Fowl.	*Material.*	*Dose and Route.*	*Date.*	*Results.*	*Pure Virus.**	*Date.*	*Results.*	*Remarks.*
Susceptible Fowl No. 359	2 c.c. Philippine immune serum 1 c.c. saliva Newcastle virus 1 day in ice-chest	1·5 c.c. of mixture. Intramuscularly	22.6.29	Remained normal	1 c.c. fresh, pooled Newcastle virus subcutaneously	5.7.29	Remained well	It appears that fowl had been vaccinated by the serum - virus mixture.
Susceptible Fowl No. 360	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto
Susceptible Fowl No. 361 Control	2 c.c. distilled water. 1 c.c. saliva, Newcastle virus 1 day in ice-chest	1·5 c.c. of mixture. Intramuscularly	Ditto	Died, 27.6.29. Symptoms typical. Lesions good	—	—	—	Showing saliva used was virulent.
Susceptible Fowl No. 362 Control	Ditto	Ditto	Ditto	Remained normal	1 c.c. fresh pooled Newcastle virus, subcutaneously	5.7.29	Remained normal	Fowl may have been immune, or vaccinated.

*This virus is shown to be virulent from Fowls No. 354 and 355 ; see Set No. VI.

TEST VI.

NEUTRALISATION OF NEWCASTLE VIRUS BY PHILIPPINE IMMUNE SERUM.*

Fowl.	*Material.*	*Dose and Route.*	*Date.*	*Results.*	*Test of Immunity.*	*Date.*	*Results.*	*Remarks.*
Susceptible Fowl No. 353	1 c.c. Philippine immune serum. 1 c.c. Newcastle virus suspension. 1 day in ice-chest	1 c.c. mixture intramuscularly	28.6.29	Remained normal	Fresh pooled Newcastle virus	5.7.29	Became sick but recovered	—
No. 354	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Became sick, paralysis developed. Died 13.7.29	Symptoms and lesions were not different from Philippine disease.
No. 355	5 c.c. Philippine immune serum. 1 c.c. Newcastle virus suspension. 1 day in ice-chest	3 c.c. of mixture intramuscularly	Ditto	Ditto	Ditto	Ditto	Became sick. Symptoms typical. Lesions good. Died 12.7.29	Ditto
No. 356	Ditto	Ditto	Ditto	Ditto	Ditto	Ditto	Became sick but recovered	—
No. 357 Control to serum	5 c.c. normal fowl serum. 1 c.c. Newcastle virus suspension. 1 day in ice-chest	3 c.c. mixture intramuscularly	Ditto	Became sick. Symptoms typical. Lesions good. Died 3.7.29	—	—	—	—

*Suspension of spleen, brain, liver and kidney was filtered through paper to avoid large particles. Virus was then accessible to the action of antibodies in the serum.

TEST VI (*Continued*).

Fowl.	*Material.*	*Dose and Route.*	*Date.*	*Results.*	*Pure Virus.*	*Date.*	*Results.*	*Remarks.*
No. 358 Control to serum	5 c.c. normal fowl serum. 1 c.c. Newcastle virus suspension. 1 day in ice-chest	3 c.c. of mixture intramuscularly	28.6.29	Became sick. Symptoms typical. Lesions good. Died 4.7.29	—	—	—	—
No. 370 Control to virus	Pure Newcastle virus in distilled water	0·5 c.c. of suspension intramuscularly	Ditto	Became sick. Symptoms typical. Lesions good. Died 3.7.29	—	—	—	—
No. 371 Control to virus	Ditto	Ditto	Ditto	Became sick. Symptoms typical. Lesions good. Died 3.7.29	—	—	—	—

Cross-Immunity Tests with Newcastle Virus and the Philippine Virus carried out at Weybridge.

Philippine Virus.

Received from Dr. E. A. Rodier, Philippine Islands, and inoculated as follows :—

Fowl No. 775 inoculated intravenously with 1 c.c. of spleen suspension. *No reaction.*

Fowl No. 718 inoculated intramuscularly with 2 c.c. of brain suspension. *No reaction.*

Fowl No. 701 inoculated intravenously with 1 c.c. of saliva in glycerine-saline. Died 5th day.

Experiment I.

This experiment was carried out in iron cages measuring 3 ft. × 3 ft. × 3 ft.

Cage I.

Newcastle Immune Fowl 589 inoculated intravenously with 1 c.c. suspension of spleen of No. 701 (Philippine virus). *No reaction.*

Healthy Fowls 679 *and* 634 inoculated intravenously with 1 c.c. of same material, died on the 5th and 6th day respectively.

Newcastle Immune Fowl 506 placed in cage with 589, 679, 634. *Remained healthy.*

Healthy Fowl 630 placed in cage with 589, 679, 634, 506, died on 7th day.

Cage II.

Newcastle Immune Fowl No. 570 inoculated intravenously with 1 c.c. suspension of spleen of No. 701 (Philippine virus). *Remained healthy.*

Healthy Fowl 625 inoculated intravenously with 1 c.c. of same material. Died on 4th day.

Healthy Fowl 633 placed in cage with Nos. 570 and 625. Died on 11th day.

The results obtained in this experiment prove that fowls hyper-immunised against Newcastle virus resist infection with the Philippine virus.

Experiment II.

Fowls Nos. 775 and 718, which had shown no reaction when inoculated with spleen and brain suspension received from Dr. Rodier, were re-inoculated 17 days later with Philippine virus and resisted infection. A control fowl, No. 873, inoculated with the same material died on the fifth day.

Fourteen days later Nos. 775 and 718 resisted, without any apparent effect, an intravenous inoculation of the Newcastle virus ; a similar dose of the same material killed a control fowl in five days.

Although the number of birds in this experiment was small, the results showed clearly that birds immune to the Philippine virus resist infection with the Newcastle virus.

Experiment III. Serological tests.

Newcastle Immune Serum.

This serum was obtained from fowls hyperimmunised against Newcastle virus.

Newcastle virus. Mouth exudate.
Philippine virus. Mouth exudate.

The serum and virus were mixed in equal parts (1 c.c.) and kept in the dark at room temperature (14° C.) for four days, and 1 c.c. of the mixture was then inoculated intravenously into fowls. Controls of both viruses were put up with equal parts of normal saline solution and also with equal parts of normal fowl serum.

Tube I.—Newcastle virus + Newcastle immune serum. Inoculated fowl remained healthy. The virus was completely neutralised by the serum.

Tube II.—Newcastle virus + normal fowl serum. Inoculated fowl died on the 6th day.

Tube III.—Newcastle virus + normal saline solution. Inoculated fowl died on the 6th day.

Tube IV.—Philippine virus + Newcastle immune serum. Inoculated fowl remained healthy. The virus was completely neutralised by the serum.

Tube V.—Philippine virus + normal fowl serum. Inoculated fowl became very sick, but eventually recovered.

Tube VI.—Philippine virus + normal saline solution. Inoculated fowl died on the 5th day.

These experiments, carried out at Manila and at Weybridge, proved the Philippine and Newcastle viruses to be immunologically indistinguishable.

We made tests on similar lines with Newcastle and Java viruses and found them to be indistinguishable.

Picard obtained identical results with the Java, Philippine and Newcastle viruses.

Cooper found that the Indian (Ranikhet disease), Philippine and Newcastle viruses were immunologically indistinguishable.

Nakamura, Oyama, Fukosho and Tomonaga (1933) studied the relationship between the virus of the Korean poultry disease, the Philippine virus, Newcastle virus, and a fowl virus isolated by Sato in Japan.

They carried out cross-immunity tests and serum-virus neutralisation tests and found that the four viruses were immunologically indistinguishable.

They state that the diseases induced by these viruses can be distinguished by their clinical symptoms, *post-mortem* lesions and cross-immunity tests from the " Chiba virus," which they consider to be identical with the virus of fowl plague. They add that if the " Chiba virus " is identical with the classical fowl plague, then their results are not in agreement with those reported by Manninger (1932).

In view of these results obtained by different workers it is reasonable to conclude that the disease observed in England,

Dutch East Indies, Philippine Islands, India, Korea, and Japan is caused by immunologically identical strains of the same virus.

Infectivity of the Blood.

Manninger (1932) carried out some comparative experiments with the viruses of Newcastle disease and fowl plague.

The Newcastle virus, which he obtained in 1930 from this Institute, was the original strain isolated in 1926, and it had been propagated since by passage through fowls.

When the virus was first isolated and during the period it was under study here no artificially infected fowl ever recovered, but the virus weakened considerably with the passage of time, and when received by Manninger it killed only a percentage of inoculated fowls.

Manninger questions the accuracy of the statement (Doyle, Kraneweld, Picard, Rodier, Edwards, Cooper and Farinas) that the virus is either absent or present only in minute amounts in the blood of infected fowls.

He claims that by ten serial passages of the virus through fowls, using as an inoculum blood taken from artificially infected fowls during the febrile reaction, he succeeded in exalting its virulence until it was capable in a dose as low as 10^{-6}g. of killing a fowl in 48 hours.

It is important to note that Manninger states that parallel with the increase in virulence of the virus the clinical picture and the pathological lesions exhibited by affected fowls gradually changed and after a few passages became indistinguishable from those of classical fowl plague.

It was the fact that the inoculation of blood from naturally infected fowls frequently failed to produce the infection and that the period of incubation in those fowls that became affected was considerably longer than is usually the case in fowl plague, which first aroused suspicion that the disease at Newcastle was not the classical fowl plague.

The blood of a fowl that has died of fowl plague invariably causes the infection when inoculated into a susceptible fowl.

We have on many occasions taken blood from fowls at different stages of the disease, but have not found any evidence that the virus content was appreciably higher at one period than another; sometimes the test fowls contracted the infection, but more often they remained normal and on subsequent immunity test proved susceptible.

Kraneweld never succeeded in setting up the disease in susceptible fowls by the inoculation of blood from fowls infected with the Java virus.

Picard tested the infectivity of blood drawn from fowls during the incubative stage of the disease, from those exhibiting clinical symptoms (diarrhœa, dyspnœa, and nervous symptoms) and from

fowls recently dead of the disease, but he failed to set up the infection.

He obtained negative results also from the intravenous inoculation of large amounts of whole blood, defibrinated blood, washed blood corpuscles and the sera of affected fowls.

Rodier states: "Fowl plague is transmitted by minute quantities of blood to healthy fowls, but this disease (Philippine fowl disease) is not so transmitted."

Edwards, in an early report on "Ranikhet disease," refers to the non-inoculability of the virus with the blood of affected fowls.

Cooper found that the inoculation of blood sometimes failed to produce infection.

Farinas failed to set up infection by intravenous and by subcutaneous inoculation of whole blood, blood serum, and washed red blood corpuscles. The transfusion of blood from affected to healthy fowls was successful in two instances in setting up the disease.

In view of these results, obtained in England, Dutch East Indies, Philippine Islands and India, it is a remarkable and significant fact that Manninger should have encountered no difficulty in inducing infection by the inoculation of minute quantities of blood.

The Effect of the Rapid Serial Passage of Newcastle Virus through Fowls and Pigeons.

We have carried out an experiment on similar lines to that of Manninger in order to confirm his statement that the virulence of Newcastle virus can be exalted, and the duration of the disease reduced, by the rapid serial passage of the virus through fowls, using as an inoculum blood taken during the early febrile period of the infection.

We were immediately confronted with the fact that in some of the fowls only a slight rise of temperature occurred and it was difficult to decide the optimum time to bleed them.

To overcome this obstacle we passaged the virus at intervals of 72 hours, which appears to be about the period when the most constant rise of temperature occurs in fowls that show a febrile reaction.

The passage fowls were killed at the seventy-second hour, and 1·0 c.c. of heart blood inoculated intravenously into another fowl. The virus was passaged 15 times in this manner. A control fowl was at each passage inoculated intravenously with 1·0 c.c. of the same material. The control fowls were allowed to die in order that any change occurring in the virulence of the virus could immediately be detected.

In addition 1·0 c.c. of the virus from each passage was inoculated intravenously into a young pigeon.

The results of the experiment are shown in Table No. I.

TABLE I.

THE RAPID SERIAL PASSAGE OF NEWCASTLE VIRUS THROUGH FOWLS AND PIGEONS.

Passage.	*Passage Fowl Number.*	*Fowl control to virulence of passage virus.*		*Effect on passage virus on Pigeons.*	
		No.	*Day of Death.*	*No.*	*Day of Death.*
1st	53	57	7th	1572	7th
2nd	54	46	6th	1573	9th
3rd	40	138	6th	1574	9th
4th	180	62	5th	1575	6th
5th	182	188	6th	1576	7th
6th	174	211	6th	1577	10th
7th	219	191	9th	1578	Ill. Recovered
8th	221	214	5th	1579	7th
9th	271	386	16th	1580	12th
10th	264	267	9th	1581	Ill. Recovered
11th	286	364	6th	1582	Ill. Recovered
12th	367	299	7th	1583	8th
13th	280	297	7th	1584	7th
14th	360	362	6th	1585	7th
15th	406	395	Ill. Recovered	1586	7th

It can be seen that the rapid serial passage of the virus brought about no appreciable change in its virulence as judged by the time taken to kill susceptible fowls. Neither did any diminution in virulence of the virus for pigeons occur as might have been expected if rapid passage rendered it indistinguishable from the classical plague virus, as claimed by Manninger.

It is of interest to note here that Cooper made 56 serial passages of the Ranikhet virus in fowls, using filtered "mouth washing," without effecting any change in either the clinical or in the pathological picture of the disease.

CROSS-IMMUNITY TESTS WITH THE VIRUSES OF NEWCASTLE DISEASE AND FOWL PLAGUE.

Manninger is apparently under the impression that we made no attempt to immunise fowls against plague virus by means of immune serum and by formalised virus. A glance at pages 165 and 166 of our previous article will show that both these methods were tried.

His criticism that we used only a small number of fowls in the cross-immunity tests is certainly strange, in view of his own experience, which confirmed our results, that it is extremely difficult to immunise fowls against the inoculation of plague virus.

We used a large number of fowls, and tried several types of vaccine, in an endeavour to obtain some sufficiently protected to withstand an intramuscular inoculation of plague virus, but with little success.

Carpano states that "a bird which had already *recovered* from an experimental infection of an integral virus naturally attenuated, may succumb to the inoculation of a small dose of highly virulent plague blood."

Purchase found that fowls immunised to withstand a lethal dose of plague virus may after a lapse of 32 days or longer succumb to an intramuscular inoculation of the homologous virus.

Beaudette (1925), Komarov (1934) and others have also recorded the difficulty of immunising fowls against plague virus.

It is of interest to note here that Todd (1928) tested four strains of plague virus, and Purchase (1931) tested seven strains, and found them to be immunologically identical.

In our experience it is less difficult to immunise fowls against Newcastle virus than it is against plague virus.

Manninger succeeded in immunising nine fowls, five against plague virus and four against Newcastle virus. In the subsequent cross-immunity tests none of the fowls died.

His statement that the fowls used by us in the cross-immunity tests were not proved to be immune to the homologous virus is, with the possible exception of one fowl, inaccurate.

We *hyperimmunised* ten fowls against Newcastle virus and subsequently tested four against artificial infection, and six against natural infection, with plague virus. *All died.*

We succeeded in immunising one fowl only to resist an intramuscular inoculation of plague virus. This fowl on subsequent test with Newcastle virus died on the eighth day after inoculation.

A second fowl that had received three subcutaneous injections of formalised plague virus resisted gross natural infection with plague for three weeks in a small cage in which 23 fowls died of the disease during the same period. This fowl when placed in-contact with a fowl affected with Newcastle disease died on the tenth day.

In addition to the cross-immunity tests we carried out serum virus neutralisation tests and concluded as follows:—

"The results obtained, however, showed clearly that anti-plague serum neutralised a very considerable part of the plague virus, but had no effect on the Newcastle virus; and that the anti-Newcastle serum completely neutralised the Newcastle virus, but had no effect on the plague virus."

Picard tested the immunity against plague virus of 20 fowls

recovered from the Java disease; some had recovered from artificial infection and some from natural infection.

None showed any immunity to the inoculation of plague virus. He concluded that on immunological and other grounds the two diseases should be regarded as separate entities.

Purchase (personal communication) states that during his work on these viruses a fowl inoculated with Newcastle virus was, owing to a misunderstanding on the part of an assistant, put into a pen containing twelve fowls hyperimmunised against plague virus and that all died of Newcastle disease.

Burnet and Ferry (1934) made a comparative study of the viruses of fowl plague and Newcastle disease, the technique employed being the inoculation of the chorio-allantoic membrane of the developing egg.

Sections of the membranes of eggs inoculated with Newcastle virus showed the principal lesion to be a patchy ectodermal proliferation followed by, or associated with, gross vacuolation of the epithelial cells, and eventual necrosis. In some areas the vacuoles contained sharply-defined acidophile bodies which appeared to be true cytoplasmic inclusions. In fowl plague the membrane showed an almost complete absence of ectodermal cells, and no inclusion bodies were observed.

Filtration studies with egg material indicated that Newcastle virus was larger (80-120 mm.) than plague virus (60-90 mm.). The Newcastle virus was found to be more resistant to photodynamic inactivation by methylene-blue than plague virus.

Burnet and Ferry state that " these differences in conjunction with the known clinical and immunological differences point to the complete ætiological independence of the two diseases."

Pigeons.

The inoculation of pigeons provides an easy and reliable method for the differential diagnosis of Newcastle and fowl plague viruses.

Pigeons of all ages are susceptible to artificial infection with Newcastle virus; young pigeons are highly susceptible, and adult birds, although slightly more resistant, can be readily infected.

The principal symptoms exhibited are drooping of the wings and paralysis of the legs, followed by death on from the sixth to the tenth day after inoculation.

Pigeons are highly resistant to artificial infection with fowl plague virus even in massive doses.

A considerable number of pigeons of various ages have been inoculated with strains of plague virus during the course of years at this laboratory and in no instance have symptoms been observed. Freese, Leclainche, Kleine and Möller, and Carpano have reported similar results.

It is strange that Manninger should have omitted to employ

pigeons for differentiating between the two viruses and also for controlling the purity of his fowl plague virus. Did the Newcastle virus, the virulence of which he claims to have exalted by rapid serial passage through fowls, lose its pathogenicity for pigeons?

In view of his contention that Newcastle virus is an attenuated strain of plague virus, the following experiment has been carried out in an attempt to adapt plague virus to pigeons.

Pigeons 13 *and* 14 were inoculated intravenously with 1 c.c. of plague virus ; 0·1 c.c. of this virus killed a susceptible fowl in 40 hours.

Pigeon 13 was killed at the 24th hour and 1 c.c. of the heart blood was inoculated into :—

Fowl 1114. Died within 48 hours.

Pigeon 16. Remained healthy.

Pigeon 14 was killed at the 48th hour and 1 c.c. of the heart blood inoculated in tramuscularly into :—

Fowl 1014. Remained healthy.

Pigeon 18. Remained healthy.

As the plague virus persisted in pigeon No. 13 for 24 hours, an attempt was made to adapt it to pigeons by serial passage at intervals of 24 hours.

Pigeon No. 1. 1 c.c. plague blood* inoculated intramuscularly. Killed 24th hour.
|
Pigeon No. 2. 1 c.c. heart blood from No. 1, inoculated intramuscularly. Killed at 24th hour.
|
Pigeon No. 3. 1 c.c. heart blood from No. 2, inoculated intramuscularly. Killed at 24th hour.
|
Pigeon No. 4. 1 c.c. heart blood from No. 3, inoculated intramuscularly. Killed at 24th hour.
|
Pigeon No. 5. 1 c.c. heart blood from No. 4, inoculated intramuscularly. Killed at 24th hour.
|
Fowl No. 1012. 1c.c. heart blood from No. 5, inoculated intramuscularly. Remained healthy.

A month later fowl No. 1,012 proved susceptible to the intramuscular inoculation of 0·01 c.c. of plague virus.

The serial passage of plague virus at intervals of 24 hours failed to adapt it to pigeons and no virus was present in the blood of the pigeon of the fifth passage, as proved by its failure to set up infection when inoculated intramuscularly into a susceptible fowl.

In another experiment 20 pigeons were each inoculated intramuscularly with 2 c.c. of plague blood* and all remained healthy.

Newcastle Virus is not an Attenuated Strain of Fowl Plague Virus.

Manninger regards Newcastle virus as being an attenuated strain of plague virus, but to do so is to ignore some fundamental facts about the disease.

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*The virulence of the blood was proved by inoculation into a fowl.

First, the mortality rate in Newcastle disease from natural infection as it occurred in England and the Dutch East Indies was 98-100 per cent.

In plague the mortality rate under field conditions varies from about 20 to 100 per cent. with an average rate of about 50 per cent.

There is little difference in the mortality rate between the two viruses following artificial infection, with both it is almost invariably 100 per cent.

If, therefore, the comparative virulence of these viruses is to be judged on the mortality rate to natural infection the Newcastle virus must be regarded as being the more uniformly virulent.

Manninger claims that a small percentage only of susceptible fowls contract Newcastle disease when placed in direct contact with affected fowls, and that the degree of infectivity of Newcastle virus is similar to that of plague virus.

The following statement is taken from our previous report on this disease :—

" This is in marked contrast with Newcastle disease, in which infection always follows direct contact between a diseased and a healthy bird. Placing a healthy fowl in a cage that has recently been occupied by a Newcastle-infected bird is usually sufficient to set up the disease."

Further experience has confirmed that conclusion. Picard found that direct contact between fowls infected with the Java virus and susceptible fowls almost invariably resulted in infection. He placed 150 susceptible fowls in-contact with two infected fowls and six only failed to contract the disease; the subsequent inoculation of the six fowls with virus proved that they had either a natural or acquired immunity.

Purchase (1931) states: " From personal observation made on a number of occasions, when using Newcastle virus a contact has never been known to escape infection."

Konno, Ochi and Haschimoto showed that the " Korean poultry disease "—later proved to be immunologically identical with Newcastle disease—was readily transmitted by contact.

Fowl plague is, on the contrary, only weakly infectious in cage experiments, usually not more than about 25 per cent. of fowls contracting the disease.

Marchoux (1910), Beaudette (1925), Stubbs (1925), Krumweide and his collaborators (1925), and Meizsner and Berge (1926) failed to set up fowl plague by direct contact between infected and susceptible fowls.

The only legitimate grounds on which Newcastle virus could be regarded as being less virulent than plague virus is that the duration of the infection caused by it is longer than that caused by plague virus.

The Immunity of Fowls Recovered from Newcastle Disease.

Manninger states that fowls *recovered* from Newcastle disease are not solidly immunised against subsequent infection with the homologous virus. He records that two recovered fowls (Nos. 99 and 27) were inoculated with 10^{-4}g. and 10^{-3}g. of blood from a fowl *dead* of Newcastle disease. The fowls died in two and three days respectively.

The smallness of the lethal dose of *blood* and the short interval in both instances between inoculation and death is highly significant and is not typical of Newcastle virus.

We have had the opportunity of testing the immunity of eight fowls *recovered* from natural infection with Newcastle disease and all proved to be solidly immune.

Picard states that fowls *recovered* from artificial or natural infection with the Java virus are resistant to the intravenous inoculation of massive doses of the homologous virus, and also to contact with naturally infected fowls.

Cooper, when carrying out cross-immunity tests, found that: "fowls that had *recovered* from an attack of 'Ranikhet disease' were solidly immune to each of the other two viruses. Conversely, fowls that had *recovered* from an artificially induced attack of 'Newcastle disease' proved fully resistant to the virus of 'Ranikhet disease.'

Farinas states that fowls *recovered* from the Philippine disease are immune to subsequent infection with the homologous virus.

Temperature of Fowls Infected with Newcastle Virus.

In our original report on Newcastle disease the temperatures of two artificially infected fowls were recorded; in both cases there was a rise of temperature starting on the second day and reaching its maximum on the sixth day.

Cooper (1931) pointed out that febrile symptoms rarely occur in fowls infected with the Ranikhet virus. He observed that the first symptom was a sudden fall in temperature followed by the usual symptoms of illness; in a small percentage of fowls there was an initial rise of temperature.

Farinas states that the disease in the first stage is characterised by fever and that this is followed by an irregular or sub-normal temperature.

We have made some further observations on the temperatures of artificially infected fowls and found that the majority of them show a febrile reaction, although in some the reaction is slight, and in a small number the temperature remains normal until just before death, when it becomes sub-normal. We have not observed the preliminary fall in temperature reported by Cooper.

We are indebted to Mr. N. Dobson of this Institute for temperaturing some fowls artificially infected with the strain of

Newcastle virus that he isolated from the 1933 outbreak in this country. This strain was immunologically indistinguishable from the strain isolated in 1926 at Newcastle.

In ten fowls the rise in temperature varied from 0·8° F. to 3·2° F.; the average increase was 1·9° F.

Discussion.

Manninger apparently desires to include Newcastle disease and fowl plague under one head mainly because of their superficial resemblance, and he would appear content to ignore many fundamentally important points of distinction between them.

Such a procedure would be not only scientifically unsound and impracticable, but would obviously tend to confusion in diagnosis and consequently lead to errors in the appropriate methods of control.

There is no evidence that plurality of virus strains occurs in fowl plague, similar to that which has been established for the virus of foot-and-mouth disease.

In foot-and-mouth disease the periods of incubation, symptoms, lesions and pathogenicity for other species are similar for the three recognised strains of virus.

There is a marked resemblance in the clinical pictures set up by the viruses of vesicular stomatitis (in bovines and pigs) and foot-and-mouth disease, but these diseases have been proved to be separate entities.

The infections caused by the psittacosis virus and by the virus of parrots and parrakeets isolated by Pacheco and Bier (1930) are clinically indistinguishable, yet these viruses have been proved to be unrelated to one another.

The infection caused by Newcastle virus is distinct from that caused by plague virus in its period of incubation, symptoms, lesions, infectivity and pathogenicity for pigeons. The two viruses are also immunologically distinct.

Most of Manninger's findings on Newcastle disease are at variance with those obtained by other investigators. His statement that Newcastle virus is an *attenuated* strain of plague virus is based on frail and insufficient data and does not stand critical examination.

His claim that the rapid serial passage of Newcastle virus through fowls reduces the period of incubation and gradually renders the infection indistinguishable from fowl plague could not be confirmed.

The inoculation of pigeons provides an easy and reliable method of differentiating the two viruses, but Manninger made no use of them and he offers no explanation to account for their susceptibility to one virus and their marked resistance to the other.

He failed to carry out serum-virus neutralisation tests although these are valuable in distinguishing the viruses.

Fowl plague has been under constant observation during the past 40 years in Europe, yet there does not appear to be a single reference in the literature to the occurrence of respiratory symptoms, whereas a characteristic respiratory symptom occurs in at least 70 per cent. of fowls naturally infected with Newcastle virus.

Conclusions.

(1) Newcastle disease has been identified in England, Dutch East Indies, Philippine Islands, India, Ceylon, Korea, Japan and Australia.

(2) The English, Dutch, Philippine, Indian, Korean and Japanese strains of the virus have been proved to be immunologically indistinguishable.

(3) Newcastle disease is a separate entity, bearing a superficial resemblance to fowl plague, but differing from it in the period of incubation, symptoms, lesions, infectivity, pathogenicity for pigeons, and on immunological grounds.

(4) The wide distribution of the disease, its high infectivity and its great virulence render it a serious menace to all countries in which poultry farming is extensively practised.

(5) Manninger's contention that the rapid serial passage of Newcastle virus through fowls reduces the period of incubation and gradually renders the infection indistinguishable from plague, could not be confirmed.

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"SWELLED HEAD" OR "BIG HEAD" IN RAMS DUE TO LOCALISED INFECTION BY *CLOSTRIDIUM ŒDEMATIENS.* SEROUS, NON-GASEOUS, MALIGNANT ŒDEMA OF THE HEAD OF RAMS.[1]

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The terms "swelled head" and "big head" have been applied popularly to conditions involving œdematous swellings of the head in sheep, and sometimes goats, in various English-speaking countries of the world. The terms describe a symptom which may be common to several diseases more or less difficult to differentiate from one another.

Symptoms of this nature are to be found described in old writings dealing with diseases of sheep. Steel (1890) mentions an obscure form of "great head," possibly a specific œdema, which was described by Hogg as occurring in Skye and the Grampians among sheep which frequent the bare tops of these and other hills. It was attributed at one time to a peculiar little monster, Phaam, who frequents the mountains and, so the story goes, if any living being crosses where he has passed that being will suffer from enlarged head in mimicry of his large skull.

The disease would appear to have been rarely if ever seen in Great Britain in more recent years. In Australia, South Africa and the United States of America, œdema of the head of sheep has been observed as a symptom of one or more diseases in which the ætiology has been obscure.

Probably one of the earliest of these diseases to become defined was Geeldikkop in South Africa, being first mentioned in 1894 in the Report of the Colonial Veterinary Surgeon, Cape of Good Hope, then by Dickson (1899) and Hutcheon (1904). Paine (1906) contributed further to the elucidation of the problem and finally Theiler (1918) was able to determine its cause. To-day there are recognised at least three diseases in South Africa characterised by swelling of the head, Geeldikkop, Dikoor and Dikkopsiekte. Dikoor was described by Steyn (1928), and Dikkopsiekte, which was also known by the names "swelled head" and "big head," by deKock (1928). Geeldikkop and Dikoor occur in either sex and are the result of photosensitisation by *Tribulus* spp. in the one case, and by some unknown substance or plant in the other.

[1]The work embodied in this report was done in South Australia while the author was holding the position of Director of the South Australian Government Laboratory of Pathology and Bacteriology, Adelaide Hospital.

Dikkopsiekte, on the other hand, is confined to rams and the etiology has remained obscure.

In the United States of America œdema of the head of sheep is observed as the result of photosensitisation but the cause has remained undefined in most instances (Marsh, 1929). Jungherr (1931) has found *Agave lechequilla* to cause photosensitisation of sheep and goats in West Texas. Howarth (1934) says that "swelled head" is a non-contagious, non-infectious disease usually affecting young rams, the direct cause of which is unknown, but he draws no distinction between the disease which is said to affect young rams only and that which affects ewes and wethers.

In Australia a disease known as "swelled head" affecting young stud Merino rams has been known for many years. It came under investigation by us in South Australia in 1920 but, chiefly owing to the sporadic nature of its occurrence and the rapidity with which death usually occurs, it was not until 1928 (Bull) that its etiology was established. Sporadic cases due to photosensitisation with much œdema of the head have also been observed (Bull, 1927), but this condition may be found in ewes and weathers and can be differentiated from the characteristic disease found only in young rams. The disease is also known in other parts of Australia but has received little attention except in New South Wales. Stewart (1929) has described a typical occurrence of the disease in young rams. Other types of œdema of the head are found in New South Wales and these, along with that type confined to rams, have been studied by Seddon (1926, 1927, 1931). Seddon (1927) has been unable to find any difference in the pathology of the disease known locally as "yellows," which occurs in sheep generally, and "swelled head" of rams, but recognises differences in incidence. Later (1931) he suggests that the term "swelled head" represents more than one disease entity. Dodd (1927) was investigating a disease in sheep due to an infection by one of the members of the gas-gangrene group of bacteria but the investigation remained unfinished at his untimely death. This condition caused swelling of the head quite frequently in rams on one property but the disease was not confined to rams nor the infection to the head.

It would appear, therefore, that in the countries mentioned there is at least a popular belief that rams suffer from a specific œdema of the head, but in many instances this has not been differentiated from œdema of the head in sheep generally, due to photodynamic action in sensitised animals. Photosensitisation of sheep may be the result of ingestion of definite sensitising substances which can be demonstrated in certain plants or it may result from liver damage with jaundice, as demonstrated by Quin (1933), and circulation in the blood stream of phylloerythrin, as demonstrated by Rimington and Quin (1934). Photosensitisa-

tion of sheep may in certain instances result in severe œdema of the head, but the ears usually show a very marked reaction.

The problem has been to differentiate between the various causes of œdema of the head of sheep. Previous work has shown that this may be the result of (1) photodynamic action, or (2) infection. The present report deals only with infection as a cause of a specific and characteristic œdema confined to the entire male sheep. A preliminary report was made earlier (Bull, 1928, 1929), but the evidence on which the conclusions were based is outlined here for the first time.

Œdema of the Head due to Cl. œdematiens.

Excluding œdema of the head due to photosensitisation or to gangrenous infections, there remains a disease due to local infection of the head with *Clostridium œdematiens* which possesses characteristic features. Information obtained from various sources indicates that the disease may be found in most parts of Australia, but the following account deals only with personal observations made in the State of South Australia.

Occurrence.—The disease is confined to rams and for the most part to the Merino, although occasional cases may be seen in rams of other breeds. It occurs in young rams, usually under the age of two years, although older animals may rarely be affected. It makes its appearance in the summer and autumn, but usually more cases are to be found in the autumn than in the summer. Only a small proportion of animals become affected. On some properties the disease occurs regularly each year but on others it is irregular in occurrence. The incidence varies from the occurrence of only a sporadic case to from 2 per cent. to 4 per cent. On one property approximately 15 per cent. of the young rams were lost annually over a period of years, but such a high incidence is exceptional. In spite of the low incidence the monetary loss may be great on account of the high value of good stud rams.

It is usually found in stud flocks, where conditions are most favourable to its occurrence. It would appear that, under some circumstances, a certain amount of cultivation of the soil may favour its appearance, as it has been observed in South Australia most frequently on properties where lucerne is grown. Certain districts favour its occurrence, probably on account of the nature of the soil.

We are able, therefore, to appreciate a definite sex, age, seasonal and geographical incidence. The seasonal incidence is, at least in part, associated with the age of the animals.

Clinical.—The condition develops rapidly without premonitory symptoms. The swelling starts about the face and soon involves the eyelids, nose, lower jaw and throat, with usually some extension along the neck, even as far as the brisket. The œdema may extend to the ears but is usually slight in this situation. Respira-

tion becomes rapid and there is often evidence of nasal obstruction accompanied by a snoring noise. The body temperature may become elevated above 105° F., particularly in the early stages, and has been observed to reach 109·8° F. in one case. Fever, however, is not a pronounced feature of the disease.

As the disease progresses the œdema increases, even exuding through the skin, and the animal refuses food and water. The eyes become completely closed on account of the œdema of the eyelids and conjunctivæ. The animal usually remains in a resting position and is disinclined to get on its feet. The distress of the animal does not appear to be entirely due to the œdema about the head and obstruction to respiration, but in large part to a general toxic state. Examination will usually reveal the presence of hydrothorax. Death usually occurs in from 48 to 72 hours from the onset of symptoms. It may occur quite suddenly, particularly if the animal is being handled.

In some cases only a mild and transitory swelling of the head develops, and the animal quickly recovers. This is of rare occurrence and it is usually believed by sheep breeders that the disease is always fatal. Certainly once the œdema has become definitely well established death usually results within 48 hours.

Post-mortem Appearances.—The skin and subcutaneous tissues about the head and neck are seen to be intensely infiltrated by a clear straw-coloured fluid rich in fibrinogen and clotting readily. There may be much infiltration about the parotoid gland and the tissues of the throat, with extension along the loose tissues surrounding the trachea. The muscular tissue of the parts is normal in appearance. The fluid escapes readily on the first incision and the tissues are tense with the infiltration. The mucous membrane of the nose covering the turbinate bones is often congested and swollen. There is usually a hydrothorax involving both pleural cavities. The fluid is clear and straw-coloured. It varies in amount and is greatest if death has been delayed for 48 hours or more, when it may measure a litre or more in each cavity. There may be some œdematous infiltration of the interlobular connective tissue of the lung, particularly in the anterior lobe, and some collapse of the borders. The pericardium may contain an excess of fluid and fibrinous clots may be present. The heart is usually normal in appearance, although a few petechiæ may be found on the endocardium. The contents of the abdomen show no abnormality apart from some passive congestion of the liver. The testicles are normal.

If the wool is carefully removed from the forehead by shaving or other suitable means one or more small wounds in the skin will be seen. These are usually quite insignificant in appearance and show little or no inflammatory reaction apart from the œdema which is also present in this part.

Bacteriological Examination.—Microscopic examination of

smears of the œdema fluid fails to reveal the presence of leucocytes or bacteria and culture medium inoculated with this fluid remains sterile under ærobic or anærobic conditions. Bacteria cannot be detected by microscopic or cultural methods in the pleural or pericardial fluids nor in any of the parenchymatous organs. Material collected from one of the wounds in the skin of the forehead may reveal the presence of *Cl. œdematiens* either in pure culture or along with one or more associated micro-organisms. If the surface is carefully sterilised and material collected from the cutis or subcutis about 1 cm. from the wound a pure culture of *Cl. œdematiens* may be obtained. Material collected 2 cm. or more from the wound is found to be sterile.

The bacteriological examination is preferably carried out before the death of the animal as *Cl. œdematiens* may disappear from the tissues in the later stages of the disease and, further, invasion of the tissues by other micro-organisms may occur as an epiphenomenon. As the wounds are usually multiple it is advisable to make a bacteriological examination of each, paying particular attention to the more recent and less severe abrasions.

Bacteriology.—It is well-known that strains of *Cl. œdematiens* may differ somewhat in their cultural characteristics from the classical strain of Weinberg and Séquin. The strains isolated were characteristically toxigenic, but rather quickly lost this property under the conditions of artificial culture in cooked meat medium. When first isolated the bacillus spored readily in this medium but on subcultivation spored less readily. The spores were subterminal in position. The bacillus in young cultures was strongly Gram-positive but the strain became less strongly held in older cultures and many negative forms were to be seen. The bacilli occurred singly with no tendency to chain formation. They resembled in morphology the strains isolated from cases of necrotic hepatitis by Turner (1930), except that they were slightly smaller both in length and breadth. They fermented glucose readily, but lævulose, lactose, saccharose, galactose, maltose, dulcite, mannite, inulin and raffinose were not attacked after ten days. Growth in the media containing these substances was poor, so that little reliance can be placed on the results. Under better conditions the bacillus might be found to break down some of the other "sugars." After ten days' incubation litmus milk showed an acid reaction with some thickening, but no definite clot. There was no digestion of Loeffler's blood serum but gelatine became liquefied. The bacillus grew well in hormone broth but not so well in "v.f." broth which Turner found so satisfactory for growth of his strains and which in our hands also proved very satisfactory for his strains. Filtrates from young cultures proved toxic for guinea-pigs and the toxin was completely neutralised by type anti-œdematiens serum received from Dr. O'Brien, Wellcome Physiological Research Laboratories, and anti-œdematiens serum

prepared by Dr. Turner from " black disease " strains. The final classification of the bacillus depended on these neutralisation tests and a more complete study of the cultural characteristics of the organism has not been made.

Broth cultures showed little loss of toxicity when filtered through Berkefeld filters but much loss of toxicity when filtered through Seitz filters.

Strains were isolated from cases of " swelled head " in rams from two different properties, but experimental trials at reproducing the disease were made with only one of these strains.

Experimental Reproduction of the Disease.—A young ram, six to seven months old, showing symptoms of " swelled head," was received at the Laboratory on the night of March 21st, 1928. Slight symptoms had been observed for the first time on the morning of the same day. The animal had stood a train journey of 100 miles very well. There was tense œdema about the face, mainly involving the eyelids, which were completely closed. There was also swelling over the nasal bones, some about the lips, and a tense œdema below the lower jaw. The temperature was 103·4° F. Inspection of the head revealed a wound at the base of the right horn. The skin was broken and discoloured with some dry exudate on the surface. There was also a superficially abraded area in the centre of the forehead, extending downwards for about three inches with a small amount of blood-stained exudate on the surface. These wounds only became evident after the wool had been removed from the area. The following day the area over the forehead was shaved and the surface sterilised by the application of an iodine pad. Material was collected from the central superficial wound as well as from the subcutaneous tissues about 1 cm. and 3 to 4 cm. from the wound. This material was inoculated into cooked meat medium. Cultures were obtained from the wound and from the œdema adjacent to the wound, but the œdema from the more distant situation yielded no growth in the culture medium. The primary cultures appeared to be pure and that from the wound was used for the inoculation of experimental animals. A more rigid bacteriological examination subsequently revealed the presence of a non-pathogenic, saccharolytic, anærobic bacillus in this culture along with *Cl. œdematiens*. This will be called " original culture." Six experimental animals were inoculated with this mixed original culture. Subsequently other animals were inoculated with a pure culture obtained by plating the original culture. The culture obtained from near the wound was proved subsequently to be a pure culture of *Cl. œdematiens* and further animals were inoculated with this.

Cooked meat medium was used for the propagation of these cultures, but later this was changed for liver mash. The first subculture from the original culture was used to inoculate two

rams. One received 0·2 c.c. of the fluid portion of the 24 hours' old subculture into the subcutaneous tissues of the forehead. The skin of the forehead of the other animal was scratched with a scalpel which had been contaminated by the same culture. In 60 hours Ram I showed intense œdema of the subcutaneous tissues of the face. The eyelids were completely closed and the œdema extended to the loose skin of the lower jaw. The temperature was 105·0° F. but fell to 102·4° F. during the day. The œdema increased a little during the next 24 hours and then decreased a little on the fifth day. The ram died on the evening of the sixth day. At autopsy a clear, pale, yellow-coloured œdema was found filling the subcutaneous tissues about the face and below the lower jaw. There was also slight œdema in the subcutaneous tissues of the neck and over the sternum. The pleural cavities each contained from 1·5 to 2 litres of a clear, pale, yellow-coloured fluid. The lungs were slightly œdematous and collapsed along the lower margins. There was œdema of the tissues of the mediastinum but no increase in pericardial fluid. There was no increase in fluid in the abdominal cavity but the liver and kidneys were slightly congested. Tubes of culture medium inoculated with fluid from the pleuræ, from near the site of inoculation and from the chin remained sterile.

Ram II failed to develop any abnormality.

The "original culture" was returned to and another subculture made. Ram III was injected subcutaneously in the forehead with 0·2 c.c. of this culture. As the animal showed no departure from normal by the fifth day it was turned out and allowed to run with other sheep. On the evening of the sixth day it showed a well-developed œdema over the nasal bones, cheeks and under the lower jaw. There was no change in the skin at the site of inoculation. The animal remained bright and ate well. The œdema decreased somewhat but the animal was found dead on the morning of the twelfth day after inoculation. The *post-mortem* findings were similar to those described. The micro-organism could not be recovered from the site of inoculation nor from the œdema elsewhere.

After a series of subcultures had been made Ram IV and Ewe V were each given 0·2 c.c. of a 24 hours' old growth into the subcutaneous tissues of the forehead. These animals failed to show any departure from the normal. Ram VI was inoculated in the forehead with a needle contaminated with the same culture and after the tissues had been lightly bruised with a sterile hammer. This animal also failed to develop any abnormality.

The micro-organism had failed to retain its toxigenic power under the conditions of artificial culture. Further inoculations were carried out with a culture obtained after plating and proved to be a pure culture of *Cl. œdematiens*. This culture will be called I.C.6. Ram IIA was inoculated with 0·3 c.c. of a 24 hours

old growth subcutaneously in the forehead. Œdema of the eyelids, face and under the lower jaw developed in 44 hours. The animal died on the morning of the third day. The *post-mortem* appearances were typical but there was less fluid, only about half a litre in each pleural cavity. The micro-organism was recovered from the site of inoculation but not from the œdema 5 cm. distant or from any other region of the body.

A few days later Ewe V was inoculated in the same way with 0·2 c.c. of a subculture of I.C.6. With the same subculture Ram VIA was injected with 0·2 c.c. subcutaneously in the anterior aspect of the middle metatarsal region. Ewe V developed œdema about the face in 20 hours and the temperature was 107·0° F. The swelling increased in the next 24 hours and the eyelids became closed. The œdema remained about the same on the third day but the temperature had dropped to 102·5° F. On the fifth day the animal was moribund and was slaughtered. The *post-mortem* appearances were typical except that for the first time some œdema was detected in the perirenal tissues. Tubes of culture media were inoculated with material from the site of inoculation, from 5 cm. below the site, œdema under the chin, heart blood and fluid from each pleural cavity. No growth occurred in any of the culture tubes.

Ram VIA, inoculated with the same culture as Ewe V but into the metatarsal region, showed slight swelling about the site of inoculation in 24 hours. It was eating well, showed no evidence of pain, standing normally, and the temperature was 102° F. This swelling had completely disappeared in two days time.

Some experimental inoculations were made with cultures of the classical strain of *Cl. œdematiens* (Weinberg) received through the courtesy of Dr. Turner. The cultures were grown in liver mash for 24 hours and a dose of 0·2 c.c. was inoculated subcutaneously into the forehead of two wethers and 0·5 c.c. into a third wether. At the same time a dose of 0·5 c.c. was inoculated subcutaneously in the metatarsal region of another wether. Twenty-four hours after inoculation a slight swelling appeared at the site of inoculation in each case, but this quickly disappeared and the animals showed no further abnormality.

Further experimental inoculations were made with subcultures in liver mash of the pure culture obtained from near the wound of the natural case. The first subculture into the liver mash was incubated 48 hours when the second subculture was made and also incubated for 48 hours. A third subculture was made and incubated for 24 hours, when it was used to inoculate two wethers. One wether was given a dose of 0·25 c.c. into the subcutaneous tissues of the forehead and the other 0·4 c.c. into the metatarsal region. In 44 hours the first wether showed œdema extending over the nasal bones and slight œdema of the tissues of the forehead which did not extend and disappeared on the fourth day.

The animal inoculated in the metatarsal region developed subcutaneous œdema about the site of inoculation and extending upwards to the hock in 44 hours, but this disappeared by the fourth day.

Further inoculations were made with this culture from near the wound, but instead of using the third or fourth subculture for inoculation a single subculture into liver mash was made and incubated for 24 hours. Wether XIII was inoculated with 0·4 c.c. into the forehead after light bruising of the tissues with a hammer. In 24 hours slight swelling over the nose and in the eyelids had occurred and the temperature was 106·4° F. The swelling increased and the temperature rose to 107·1° F. but fell to 104·8° F. the following day. The animal died in three and a half days and the *post-mortem* appearances were typical. The same method was used for the inoculation of wether XIV, the dose being 0·3 c.c. Wether XV was inoculated with 0·3 c.c. of the same culture but received no preliminary bruising. Both animals developed typical œdema of the head and died on the third day. The bruising of the tissues did not appear to facilitate the process in any way.

These experimental inoculations are summarised in Table I. Rams were used as experimental animals in the first place in order to obtain optimum conditions if sex should be a factor. It was found, however, that ewes and wethers were equally susceptible. The experimentally-produced disease resembled the natural disease in almost every detail. In some cases death was delayed rather longer than in natural cases in the field. In some of the experimental cases small areas of aseptic necrosis on the surface of the liver were found. These have not been found in natural cases. The same type of lesion was also observed by Turner in guinea-pigs inoculated with " black disease " strains. The inability to recover the micro-organism from the site of inoculation in several of the experimental cases is significant and possibly explains the failures, observed by all workers, to find a bacterium in natural cases, particularly when the bacteriological examination has been left until after the death of the animal.

There is some slight evidence in the experimental inoculations that the tissues of the forehead are more favourable to the growth of *Cl. œdematiens* than connective tissues elsewhere, such as are present in the metatarsal region. When more than one animal has been inoculated on the same day with the same culture in identical situations in the body the results have been identical. Ewe V and Ram VIa were inoculated on the same day with the same culture but the sites differed. Ewe V developed a progressive œdema of the head and died on the fifth day, while Ram VIa developed only a transient œdema of the metatarsal region with complete resolution on the fourth day.

Prophylactic Inoculations.—In order to prepare a vaccine with

TABLE I.

SUMMARY OF EXPERIMENTAL INOCULATIONS.

Exp. Animal. Date.	*Dose and Method of Inoculation.*	*First Symptoms and Result.*
Ram I ... 27.3.28	0·2 c.c. Subcutaneously in forehead	L.S. 60 hours. †6th day
Ram II ... 27.3.28	Scratch wound in forehead	No abnormality developed
Ram III ... 29.3.28	0·2 c.c. Subcutaneously in forehead	L.S. 6th day. †12th day
Ram IV ... 11.4.28	Do.	No abnormality
Ewe V ... 11.4.28	Do.	Do.
Ram VI ... 11.4.28	Forehead bruised, pricked *c.* needle	Do.
Ram IIa ... 24.4.28	0.3 c.c. I.C.6. Subcutaneously in forehead	L.S. 44 hours. †3rd day
Ewe V ... 28.4.28	0·2 c.c. I.C.6. Subcutaneously in forehead	L.S. 20 hours. †5th day
Ram VIa ... 28.4.28	0·2 c.c. I.C.6. Metarsal region	L.S. 24 hours. R. 4 days
Wether VII 19.7.28	0·2 c.c. " W." Metatarsal region	L.S. 24 hours. R. 3 days
Wether VIII 19.7.28	0·2 c.c. " W." Subcutaneously in forehead	Do.
Wether IX... 25.7.28	0.5 c.c. " W." Subcutaneously in forehead	Do.
Wether X ... 25.7.28	0·5 c.c. " W." Metatarsal region	Do.
Wether XI ... 4.8.28	0·5 c.c. N.W. Subcutaneously in forehead	L.S. 44 hours. R. 3 to 4 days
Wether XII 4.8.28	0·4 c.c. N.W. Metatarsal region	Do.
Wether XIII 28.8.28	0·4 c.c. N.W. Subcutaneously in bruised forehead	L.S. 24 hours. †3·5 days
Wether XIV 8.9.28	0·3 c.c. N.W. Subcutaneously in bruised forehead	Do.
Wether XV... 8.9.28	0·3 c.c. N.W. Subcutaneously in forehead	Do.

L.S. = local swelling : † = death : I.C.6 = culture from isolated colony : R = resolution : " W " = *Cl. œdematiens*, Weinberg : N.W. = culture from near wound.

reasonably high antigenic content some attention was given to the best method of preparing toxins of the bacillus. Most consistent results were obtained by growing the micro-organism in hormone broth containing 0·2 per cent. of glucose. This was inoculated heavily with a young growth in meat medium and incubated for 24 hours under strict anærobic conditions. Growth was rapid and precipitation of the bacillary bodies always occurred when a good toxin resulted. As the strains isolated from natural cases of the disease proved troublesome in the loss of toxigenic power, most of the work was done with a strain, B.D.19, kindly supplied by Dr. Turner. The bacterial bodies were collected in the centrifuge and the supernatant fluid was filtered through a

Berkefeld candle. In the early stages formalin to a concentration of 0·3 per cent. was added to the toxin, which was then placed at 37° C. This concentration of formalin at 37° C. always gave complete detoxification in 24 hours. The bacterial bodies were treated separately in a concentration of 0·5 per cent. formalin. The two were added together and attempts were made to immunise guinea-pigs with the mixture used as a vaccine. After from two to five injections of this mixture over a period of six weeks the guinea-pigs exhibited little or no resistance to small doses of young cultures of the micro-organism.

As the toxin lost toxicity so rapidly at 37° C. when treated in this manner, smaller concentrations of formalin were tried, even as low as 0·01 per cent., but in 24 hours 1 c.c. doses of the toxin always failed to kill standard guinea-pigs and usually there was little or no reaction to the dose. Toxic filtrates kept at 37° C. without formalin were found to become non-toxic, or almost completely so, in 24 hours. Lower temperatures and different concentrations of formalin were tried and eventually it was found that a 0·2 per cent. concentration detoxified the filtrates in from seven to ten days at 25° C.

In order to make a comparative test of the antigenic value of toxoid prepared in various ways when used with or without the addition of the bacterial bodies, guinea-pigs were injected with five preparations all made from the same culture. Group I received a vaccine prepared by adding 0·3 per cent. of formalin to the toxic culture, which was then kept at 37° C. for ten days. Group II received toxoid produced by adding 0·2 per cent. of formalin to the toxin, which was then kept at 25° C. until detoxified. Group III received toxoid produced by keeping the toxin at 37-38° C. for 24 hours. Group IV received the toxoid produced at 25° C., to which was added the bacterial bodies killed with 0·5 per cent. formalin. Group V received the toxoid prepared at 37-38° C., to which the bacterial bodies were also added. Two inoculations of 1 c.c. were given at 14 days interval and the immunity was tested a month after by inoculating each with a multiple of a M.L.D. of young culture diluted in broth. Table II gives the results of the experiment. The number of animals used was too small to supply a complete answer to the enquiry, particularly considering the wide individual variation shown by guinea-pigs in ability to respond to antigens of this type. The results, however, do suggest (1) that toxoid prepared at 25° C. with 0·2 per cent. concentration of formalin is a better antigen than that prepared at 37° C. with a higher concentration of formalin; (2) that toxoid prepared at 37° C. without formalin has low antigenic power; and (3) that bacterial bodies do help to protect when used as a vaccine under the conditions of the test. An opportunity did not occur to pursue the problem any further.

TABLE II.

Immunisation of Guinea-pigs with various Antigens.

Animal.	Test Dose of Culture.	Group I. "Anaculture," 0·3 per cent. Formalin and 10 Days at 37° C.	Group II. Toxin detoxicated at 25° C. c. 0·2 per cent. Formalin.	Group III. Toxin detoxicated at 37° C. for 24 Hours.	Group IV. Toxoid as for II, plus Bacilli.	Group V. Toxoid as for III, plus Bacilli.
A	3 M.L.D.	†72 hours	—	†60 hours	—	S.O. — R.
B	10 M.L.D.	—	S.O. — R.	†16 hours	S.O. — R.	S.O. — R.
C	20 M.L.D.	†12 hours	S.O. — R.	0	S.O. — R.	S.O. — R.

† = death : — = No reaction : S.O. — R. = Slight œdema with recovery.

A vaccine was prepared for use in the field by growing the bacillus in hormone broth containing 0·2 per cent. of glucose for 24 hours, separating the bacterial bodies from the toxin in the centrifuge and treating them with 0·5 per cent. formalin. The toxin was detoxified by adding formalin to a concentration of 0·2 per cent. and keeping it at 25° C. until a dose of 1·0 c.c. failed to produce any reaction when injected subcutaneously into the guinea-pig.

The first field trial of vaccination was made before this method of preparation was finally evolved. This was carried out in February, 1929, when 30 young rams were inoculated with 5 c.c. of a vaccine consisting of toxoid prepared by detoxifying at 37° C. for 24 hours without the addition of formalin, to which formalin-killed bacilli were added. A second dose of 5 c.c. was given three weeks later. In the interval between the inoculations one of the rams died of " swelled head," and another some months later, but no further deaths occurred in this group. Four deaths from the disease occurred in 30 unvaccinated animals left as controls.

The results of this trial were considered to be reasonably satisfactory, but a more potent vaccine was desirable.

A second field trial was carried out in September of the same year, when 78 ram lambs about five months old were inoculated with 4 c.c. of the vaccine as finally evolved. A second dose of 5 c.c. was given two months later in November, and a third in December a fortnight after the second. The results of this vaccination were more satisfactory than the first, as no case of " swelled head " developed in the vaccinated animals, although approximately 6 per cent. of unvaccinated animals died of the disease.

It was considered desirable to vaccinate the rams when about four or five months old, as the disease quite frequently is seen in rams about six months old. The first inoculation should be given as early as possible. This need not be a large dose but should be regarded as a sensitising dose, and an interval of from

one to two months should be left before the second dose. The third dose may be given a fortnight later. With the use of a smaller sensitising dose and a reasonably long interval between it and the main immunising doses a more satisfactory antitoxic immunity is established. An occasional animal may be lost from the disease, however, before immunity can be established.

Subsequent vaccinations on this property were performed by the owner. Control unvaccinated animals were not provided as the owner was not prepared to stand the possible loss. The vaccine used was prepared commercially and was the same as used in the control of "black disease" (necrotic hepatitis) as recommended by Turner. Although this vaccine has given good protection up to the present time a small proportion of animals, up to 2 per cent., has been lost. We do not think that the commercial vaccine is quite as strong antigenically as that prepared by us. We have no definite proof of this and it is possible that most of the losses have occurred in the younger animals before immunity could be established. We cannot arrive at a definite conclusion as our information is incomplete.

In our own experimental observations in the field the disease was brought under control on this one property by the use of three doses of a vaccine containing toxoid prepared by adding 0·2 per cent. of formalin at 25° C., with the addition of bacilli killed by 0·5 per cent. of formalin. This result supplied evidence in support of the conclusion reached: that "swelled head" of rams, characterised by a non-hæmorrhagic œdema, is due to infection by *Cl. œdematiens*.

Discussion and Summary.

The fact that the disease is confined to the young entire male animal, which is well illustrated in Stewart's field experiments, was difficult to explain. With the demonstration of the fact that we are dealing with an infection of small wounds about the head, this difficulty has been explained. Young Merino rams in particular are very combative and inflict minor or major injuries on one another. This we regard as the sole explanation of the definite sex incidence of the disease. Older rams do fight and do occasionally develop the disease. Their relative resistance is partly explained by the development of an immunity with increased age and partly to the fact that they are rarely kept together in such large numbers as are the younger animals. Ewes may develop the disease on properties where it is found in rams if they receive a chance injury about the head. We have seen one such case in a young ewe. Wethers are rarely kept on such properties.

The seasonal incidence is partly explained by the fact that the ram lambs are developing aggressive characteristics as they become more mature and possibly partly due to the dry weather

and increased soil contamination of the fleece. Wounds of the head of rams may be infected by other types of anærobic micro-organisms of the gas gangrene group or by more virulent and less toxigenic strains of *Cl. œdematiens*. In these cases there may be well-developed œdema of the head, but there is usually little difficulty in differentiating these infections from those due to toxigenic strains of *Cl. œdematiens*. It may not be possible to recover the bacillus when infection has been produced by a toxigenic strain of *Cl. œdematiens*, but this difficulty may be overcome in part by making bacteriological examinations as early as possible and before the death of the animal. We believe it is this difficulty in recovering toxigenic strains that has been responsible for the etiology of " swelled head " in rams remaining obscure in the several countries where it is known. We do not know of any other cause that is likely to explain the occurrence of non-hæmorrhagic œdema of the head confined to the entire male. From the published descriptions we would expect Dikkopsiekte in South Africa to have a similar origin. Most instances of œdema of the head due to photodynamic action should be capable of being differentiated from the œdemas of infective origin. With photodynamic action there is usually more œdema of the ears, which droop downwards, sometimes well-developed conjunctivitis, and usually collapse from shock, and recovery may be the rule or at least more frequent than from infection. When recovery takes place diagnosis becomes more definite with the development of necrotic lesions in the ears and superficial epithelium of the face, which lesions are quite characteristic and never develop in recovered cases from infective œdema. Further, when photo-sensitisation is the cause of similar symptoms many more cases may be seen at any one time in either sex.

Prophylactic vaccination offers a safe and reliable means of control where the incidence of the disease is sufficiently high to warrant its use. The use of toxins and cultures modified by formalin has given fairly satisfactory results in immunising against *Cl. œdematiens* infections. More knowledge is required, however, on the effect of various concentrations of formalin on the antigen. It is possible also that immunity to *Cl. œdematiens* infections is not purely an antitoxic immunity and that results may be improved by increasing the resistance to " O " antigen as well as to the toxin.

The fact that the disease is essentially a wound infection makes it possible for other infections, particularly of the gas gangrene group, occasionally to establish themselves. Sporadic cases of gas gangrene infections of the head have been seen in rams of several different breeds and in Merino rams on properties where *Cl. œdematiens* infection of the forehead is, apart from such occasional cases, the cause of " swelled head." It is possible that on certain properties the infections may be commonly mixed

infections or commonly infections by a pathogen such as *Cl. chauvœi*. Sporadic occurrences of this sort may be responsible for deaths in animals immunised against *Cl. œdematiens* only. It is essential that all outbreaks of the disease be carefully investigated bacteriologically, and where it is found that other pathogens are playing a part prophylactic measures will have to be adjusted accordingly.

Gas gangrene infections of the head have in our experience been quite uncommon. The wounds inflicted on one another by young rams fighting are not of the type that usually predispose to such infections. It would appear, however, that *Cl. œdematiens* can establish itself more readily in these superficial and relatively insignificant wounds. For the purpose of this discussion we have spoken of *Cl. œdematiens* as being outside the gas gangrene group because it produces neither gas nor gangrene, or at least the toxigenic strains do not, in the tissues.

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A POSSIBLE CASE OF CONGENITAL JOHNE'S DISEASE.

By G. W. Dunkin.

(From the National Institute for Medical Research, Farm Laboratories, Mill Hill, London.)

It has not infrequently been stated that in some instances the most logical explanation for the undiminished occurrence of cases of Johne's disease, especially on self-contained farms where importation of other cattle is either rare or unknown, is that the disease may be congenital.

Alexejeff-Goloff (1929) recorded that he had isolated the causal organism of the disease from a fœtus *in utero* and also that he had had similar success when examining some three samples of milk from cows known to be affected. There is therefore some scientific support for the current opinion existing in certain localities that congenital Johne's disease is at least a possibility.

On March 3rd, 1931, the author was presented with a pedigree Jersey cow, from a tubercle-free herd, in a more or less advanced state of Johne's disease. It was stated that she was pregnant and was due to calve in a few days. Her condition at that time did not suggest that this was the case, but nevertheless she gave birth to a bull calf on March 10th—seven days after admission.

On arrival the cow was placed in one of three isolation boxes kept for the purpose, and the calf when born was not allowed to come into contact with any unwashed part of his mother's exterior, nor was he allowed to touch the ground. Immediately after birth he was removed to a loose-box which had never housed an infected beast.

The mother never recovered and was destroyed *in extremis* 14 days after parturition had taken place. A *post-mortem* examination was made immediately after death. The cæcum, ileum and practically the whole of the intestines down to the rectum were affected with Johne's disease. The associated glands were enlarged and dropsical. All the other organs were macroscopically normal and there was a complete absence of macroscopic evidence of tuberculosis. In the light of after events now to be recorded it is regretted that a minute examination of the genital organs did not take place.

It was hoped that by taking the precautions mentioned it might be possible to rear the calf and ultimately make use of him as a stud bull, his pedigree being such as warranted this decision.

In his early days the calf was fed entirely on milk from one of our own cows, all of which are free from tuberculosis and Johne's disease. About this time one of these had recently calved and she was used as a foster-mother for the calf now under discussion. He grew quite normally and when he was one month

old was turned out to grass each day with other stock of the same age. At three months of age there was little to choose in the way of size and general appearance between this animal and others of the same age of which there were four at that time.

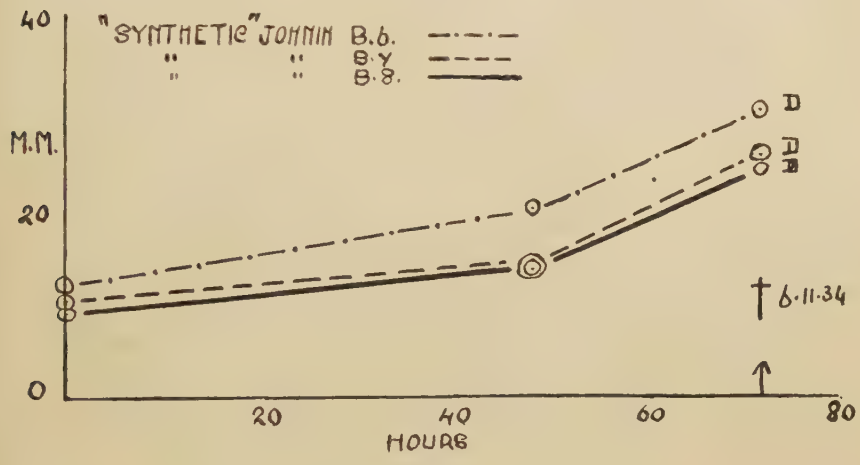

Fig. 1.

He was tested with johnin of known potency at this period and gave a pronounced positive reaction. The next test was carried out at six months, and on this occasion there was a doubtful response, although it was known that the batch of johnin used was potent. At twelve months of age a positive reaction was secured. During all this time the bull had proceeded to grow, no sign of diarrhœa was seen, and his general condition was good. Examination of fæces was made at intervals of about three or four months but on no occasion were acid-fast organisms isolated. At twelve months of age he developed a slight attack of diarrhœa which lasted for two days, during which time he suffered from inappetance. He recovered completely and was feeding on the third day of the attack and on the fourth day the diarrhœa had completely ceased. Unfortunately the author was away from home at that time and an examination of the fæces was not made.

In October, 1934, his general appearance was so good that a photograph (Fig. 1) was secured. It will be seen from this that on appearance alone he could not be suspected of being a case of Johne's disease, and on the date when the photograph was taken (October 26th, 1934) he had had no attack of diarrhœa since March, 1932. One of the objects one had in mind in securing this photograph was not so much to record his good condition at this time as to demonstrate pictorially a certain lack of muscular development of the hind quarters. The muscles surrounding the femur and to a lesser extent those of the gluteal region, had never

developed as they should have done. The anatomical appearance thus shown is not uncommon in cattle and is recognised by breeders as a point to be avoided when making a purchase. It has not, so far as I am aware, ever been suggested that its cause may be disease, but rather that it is an inherent weakness and as such may be congenital. It is a state of affairs best seen in the male or in heifers or cows which are otherwise in good fat condition. The author has observed this lack of muscle development in several previous cases which have come under observation and in these there has always been a subsequent history of Johne's disease. So far as the author is aware no cause has as yet been attributed to this anatomical defect although it is well known and recognised. Doubtless a variety of causes may play a part, such as inadequate and improper feeding during early adolescence and there are probably other causes as well. Deficient diet in the present case cannot, however, have played any part since from the first it was fully realised that the bull would have to be reared under adverse conditions and he was fed accordingly. As has been stated previously, he had the advantage of some part of the colostrum of a normal cow which fortunately had calved about the time when the case now being described was born.

FIG. 2.

Unfortunately the photograph does not show the extent of the lack of development which really existed as clearly as could be wished, but it was readily apparent even to the untutored eye by observing the animal in the standing position.

It is suggested that this symptom, taken into consideration with other points of history, may be an early clinical symptom of the disease.

No further attack of diarrhœa or, indeed, indisposition of any kind was noted until October 24th, 1934, when another attack of

diarrhœa occurred. The bull was by this time three-and-a-half years old and it is to be noted that diarrhœa had not occurred between the age of twelve months and this period. An examination of the fæces was now made and no difficulty was experienced in isolating bacilli indistinguishable from those causing Johne's disease. Cultures were prepared and a growth of organisms has been secured.

A further test with three different batches of "synthetic" johnin (1934) was now made and a positive result to all three was obtained (Fig. 2).

All the isolation boxes being occupied it was decided to destroy the bull and this was carried out on November 6th, 1934 (the day on which diagnosis was arrived at from the double intradermal johnin test), 13 days after the commencement of the second attack of diarrhœa and eleven days after the photograph was taken. Diarrhœa meanwhile had been continuous and the bull had lost much flesh.

The *post-mortem* examination was carried out immediately after death. There was a complete absence of macroscopic signs of tuberculosis and as the beast had previously passed the double intradermal tuberculin test this was not greatly surprising. Lesions of Johne's disease were observed in the rectum, ileum and ileo-cæcal valve. The associated lymphatic glands were for the most part slightly œdematous.

The following organs or portions thereof were collected: submaxillary, pharangeal, hepatic, bronchial and mediastinal glands,

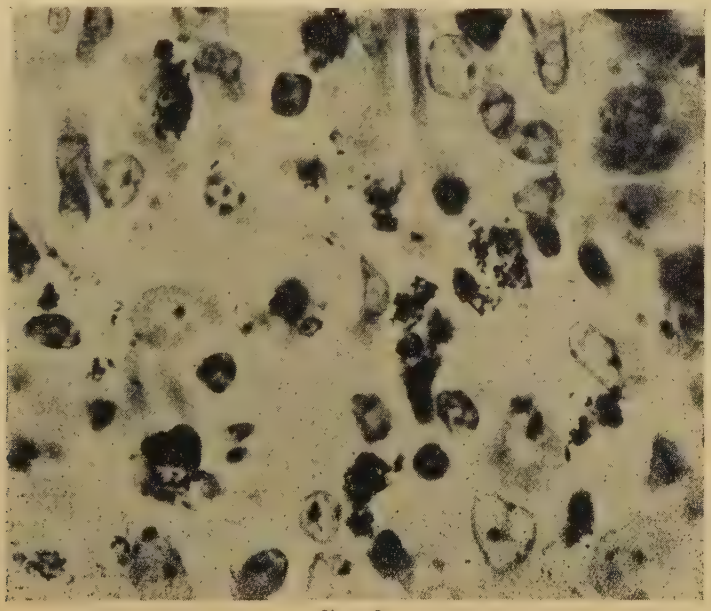

Fig. 3.

as well as certain mesenteric glands. Pieces of rectum, ileum, small intestine and testes were also secured. Direct smears were positive for acid-fast organisms in the following instances: mesenteric glands, rectum, ileo-cæcal valve, ileum and small intestine. Cultures and sections of all the above organs have been prepared.

It will be seen from the photo-micrograph (Fig. 3) of a section of the ileo-cæcal valve that the extent of the infection is severe.

The photograph of the bull illustrates his general body condition eleven days before death.

The suggestion which it is desired to convey is that the case is one of congenital Johne's disease. As against this there is no supporting evidence that infection may have been acquired on the farm, and this for two reasons. Firstly, being a bull he has been more isolated than any other bovine on the farm, and secondly, other cattle have had a much better opportunity of acquiring infection in this way since they are grazed all the year round. Again, it must be recalled that a positive reaction to the johnin test was obtained at the early age of one month. Evidence of intra-uterine infection is already to hand in the records of Alexejeff-Goleff; beyond this the author is unaware of other similar records. If, as one may suppose, intra-uterine infection is a possible method of transmission it is clear that the desirability of slaughtering the progeny of infected cows while they are still calves should be borne in mind.

I am indebted to my colleague, Mr. J. E. Barnard, for producing the photo-micrograph.

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AN ADAPTATION OF SHEATHER'S SUBSTITUTE FOR AN AUTOMATIC PIPETTE.

By H. H. Holman.

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The filling of a large number of agglutination tubes is a laborious and time-consuming process. Even when Sheather's (1928) substitute for an automatic pipette is used, there is inevitable delay between the filling of each tube owing to the time taken for the syringe to refill.

This difficulty was overcome by Beaudette (1928), who used a system employing two syringes, one filling as the other emptied.

The simple and inexpensive apparatus here described is an application of Beaudette's principle to Sheather's apparatus, and is in constant use at this Institute for agglutination tests for *Br. abortus*.

Two 2 ml. Record syringes are each fixed by spring clips to a side of two separate blood sample boxes, roughly 1-in. × 4-in. The two boxes are screwed to a wooden upright (Fig. 1A). Below the syringes, and in the centre, is a block of wood carrying a four-way metal stop-cock, with the tap bored as shown in Fig. 1B. If difficulty is experienced in obtaining a stop-cock with the necessary type of bored tap, a cheap metal four-way stop-cock

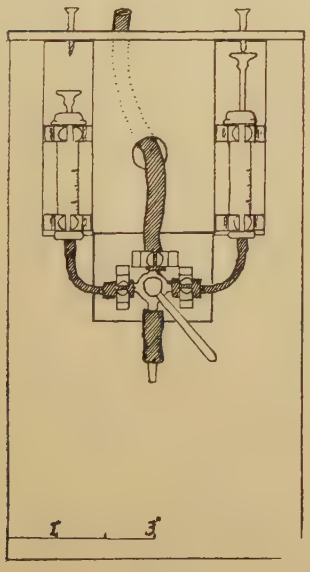

Fig. 1A.

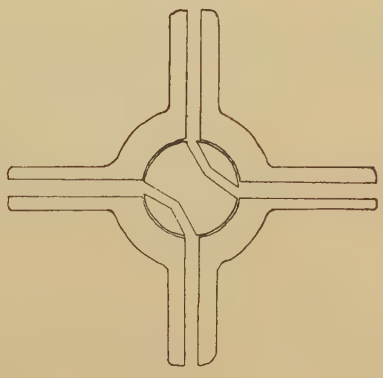

Fig. 1B.

with a simple transverse tap boring should be bought, the tap removed, and a new tap with the appropriate boring inserted. The metal seems to have no untoward action on the testing.

The four-way tap is held in position on the block of wood by three spring clips, the arms being covered with short pieces of rubber tubing to enable the spring clips to grip with sufficient pressure.

To prepare the apparatus a clip is placed on the rubber tubing leading to one syringe, and the excursion of the piston in the other syringe is adjusted to deliver 1 ml. by means of a brass screw passing through an overhanging shelf of three-ply wood. Air bubbles can be removed by pulling the syringe away from the spring clips which hold it, turning it upside down and working the plunger.

The antigen is supplied under pressure from a wash bottle fitted with a hand bellows. The time taken to deliver 1 ml. of saline into nine racks containing 126 tubes is approximately six minutes by a hand pipette, four minutes 20 seconds with Sheather's substitute, and three minutes ten seconds by use of the apparatus described.

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SOME OBSERVATIONS ON THE ISOLATION OF BRUCELLA ORGANISMS FROM RAW MILK.

By M. M. Barratt.

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Though Kennedy found agglutinins for *Br. melitensis* in the milk of English cows in 1914, and Evans in 1918 demonstrated the close cultural and serological relationship between that organism and *Br. abortus*, and it was already recognised that cattle showing clinical evidence of infection with Brucella excreted the organism in their milk the significance of these facts was scarcely appreciated in this country till in 1926 Wilson and Nutt first recorded the isolation of *Br. abortus* from ordinary market milk produced in England, and in the next two or three years reports of undulant fever originating in this country began to appear in the medical journals. Subsequent workers have abundantly confirmed the presence of the organism in milk supplies (Cruickshank and Barbour, 1931; Wood, 1931; Beattie, 1932; Morgan, 1932; Smith, 1932 and 1934; Priestley, 1932; Gaiger and Davies, 1933; Morton, 1933; Rowlands, 1933; Pullinger, 1934; and Messer, 1934) and cases of undulant fever are being recorded in increasing numbers. In these circumstances there would be little object in recording the results of yet another series of milk examinations were it not that one or two points are brought out more definitely in it than in those already published.

In October, 1931, the Institute was asked by the Medical Officer of Health of Norwich if samples of milk sent by him to the Institute primarily for the tubercle test by guinea-pig inoculation could also be examined for *Br. abortus*. Consequently a series of samples produced in the County of Norfolk were so examined in the following six months.

Technique Employed.

The centrifuged deposit of about 130 c.c. of each sample was inoculated into the thigh muscles of one guinea-pig. In the last 79 samples a portion of cream was added to the deposit. The animals were killed 29-55 days after inoculation, the majority between 29 and 37 days. The spleens of all the animals were cultured. In 94 animals cultures were also made from the superficial and deep inguinal and lumbar glands ground up together. The material was inoculated on glycerine agar slopes in 6-in. × $\frac{5}{8}$-in. test tubes. Usually a large and small inoculum was made from each tissue. Before incubation the tubes were either sealed by dipping the plugs in hot smoking paraffin or placed in a cylinder in which 10 per cent. of the air had been replaced by CO_2. In the early part of the work only sealed tubes were used

but later cultures from 56 animals were put up in duplicate, one set being sealed and the other grown in a cylinder. All the animals found to be infected in the cylinder cultures also gave positive cultures in the sealed tubes but in the case of two of the animals one of three cultures made from each remained negative in the sealed tubes though all three were positive in the cylinder. With these exceptions there was no appreciable difference in the amount of growth on the two sets but growth appeared later in the sealed tubes. As a rule even in the sealed tubes at least one culture in the set from an infected animal was positive at the end of a week but occasionally growth was first noted in an individual sealed tube as late as the eleventh day. In the latter part of the work, including rather more than half the animals, an additional culture was made from each tissue examined and grown in ordinary air. Brucella-like colonies were identified in the first place by morphology and ability or otherwise to grow in ordinary air. Those able to grow in ordinary air were tested for motility and fermentation of glucose. Cultures giving negative results in the latter tests, together with those which failed to grow in ordinary air, were examined serologically. The organism recovered from both the glands and spleens was identified. The sera of 42 of the animals were examined for Brucella agglutinins.

Results.

The milks as examined and reported were identified only by a serial number but later Dr. Soothill, Medical Officer of Health of Norwich, kindly supplied details of the types of milk represented. These are shown with the results in Table I. Unfortunately partly as a result of secondary infections there was some mortality

TABLE I.

Type of Milk.	*Number of Inoculated Animals Living 3 Weeks or More.*	*Number of Animals from which Brucella Isolated.*	*Number of CO_2 Sensitive Strains.*	*Number of Non-CO_2 Sensitive Strains.*
Ordinary	115	25 +1	21	4 +2
Ordinary from 7 herds mixed before pasteurisation ...	1	1	1	
Pasteurised	5	0	0	0
Grade A.T.T.	3	0	0	0
Certified	1	1	0	1
Single cow	28	4	2	2
Unknown	4	3	3	0
Totals	157	34 + 1 21·6 per cent.	27	7 + 2

+1 = 15 day animal. +2 = 15 day animal and the sample from which 2 strains were isolated.

among the animals, but 157 of those inoculated lived 21 days or more. Brucella was isolated from 34 of these, 21·6 per cent. In addition, cultures were made from 16 animals dying 15-20 days after injection. From one of these, a 15-day animal, Brucella was isolated. It is shown as +1 in column 4 of the table and is also indicated in column 6, but is not included in calculating the percentage. With the exception of two samples all the ordinary milk represented by these samples was subsequently pasteurised by a holder process before being sold to the public. The five pasteurised samples of milk examined came from the same retailer and were found free from Brucella. The four unknown samples were not included in Dr. Soothill's list but from their position in the series were probably ordinary milk.

The putting up of primary cultures and first sub-cultures in ordinary air showed that a comparatively large number of strains, nine of 36, were not CO_2 sensitive, that is, did not require CO_2 in primary culture or early subculture. Five of these grew freely in ordinary air in the cultures made from the guinea-pig tissues. Primary cultures in ordinary air were not made from the remaining four but these all grew well in ordinary air in the first subculture, in some cases more freely than in CO_2. It will be seen from Table I that one of the strains was found in the only sample of certified milk examined, two in single cow samples and the remainder in ordinary milk. All the single cow samples were received in the same consignment but it is not known if they all came from the same herd. Table II shows that all the infected samples of ordinary milk came from twelve of the 58 herds and that from four of these, strains not requiring CO_2 were isolated.

TABLE II.

Number of Herds from which Samples Completely Examined.	*Number of Samples from each Herd.*	*Number of Infected Herds.*	*Number of Infected Samples.*	*CO_2 Requirements of the Strains Isolated.*
33	1	3	3	All required CO_2
13	2	2	3	1 herd: 1 required CO_2, 1 did not require CO_2 1 „ 1 did not require CO_2
4	3	2	2	Both required CO_2
4	4	2	3	1 herd 1 required CO_2 1 „ 1 „ „ 1 sample both types isolated
2	5	1	3	All required CO_2
1	8	1	5	„ „
1	11	1	7	4 required CO_2 3 did not require CO_2 (includes 15 day animal)
58	116	12	26	20 required CO_2 6 did not require CO_2

From all except one of these four herds a strain or strains requiring CO_2 was recovered from a sample sent at the same time. In one sample both types were found. The eleven samples from one farm were received on three different dates. On the first, December 4th, 1931, only one of four samples was found infected and the strain required CO_2. On the second, February 19th, 1932, three of three samples were infected; two required CO_2, one did not. On April 13th, 1932, four of four samples were infected; two required CO_2 and two did not. The animal found infected 15 days after inoculation is included in the February 19th, 1932, consignment. The strain recovered did not require CO_2. On the other hand all the five strains recovered from the eight sample farm received December 18th, 1931, and February 19th, 1932, required CO_2.

Though all the glands from which Brucella were isolated showed some enlargement it was not uncommonly very slight, sometimes not greater than that to be found in almost any normal guinea-pig, there frequently being little difference on the two sides. Many of the spleens also from which positive cultures were obtained showed little if any naked-eye change. This absence of obvious lesions in many infected animals has also been noted by Cruickshank and Barbour and Gaiger and Davies.

The selection of the animals from which gland cultures were made depended almost entirely on the time available and not on the appearance of the glands. Brucella were isolated from 29 of 94 (30·8 per cent.) so examined. In the majority of animals both tissues were either positive or negative but though no positive spleen cultures were associated with negative glands on six (20·7 per cent.) occasions the gland cultures were positive when those from the spleen were negative. All except one of these six animals were killed 28-34 days after inoculation. In four of these animals only sealed cultures were put up but a good growth occurred on all the gland tubes and one of the strains isolated did not require CO_2. The cultures from the remaining two animals were grown in a cylinder and here, too, one of the strains did not require CO_2, so that in at least three animals the failure to obtain growth from the spleen could not be attributed to absence of sufficient CO_2 in the sealed tubes. The more likely explanation seems to be that either the infecting dose was small or the animals were more resistant to infection. Unfortunately the sera of only two of these animals were examined for agglutinins. One was negative in a dilution of 1 : 5 whilst the other had a titre of 1 : 40.

Including these two animals the sera of 42 were examined for Brucella agglutinins in dilutions of 1 : 20-1 : 1,280. Ten gave positive results, two in a dilution of 1 : 40, two in 1 : 320, three in 1 : 640 and three in 1 :1,280. All the positive sera were associated with positive cultures, but in one instance (noted above) the

serum was negative when *Br. abortus* was isolated from the glands but was not found in the spleen.

Discussion.

It is now generally accepted that anything from 20-40 per cent. or more of the raw milk of this country, not excepting the graded supplies, may contain Brucella in sufficient numbers to infect guinea-pigs. In this series the percentage was 21·6. No information as to the size of the herds from which these samples were sent was available but if the number of samples from each (see Table II) can be taken as a rough indication of the quantity of milk delivered and so of the size of the herds the smaller percentage of positive results when only one sample was examined, in so far as any conclusion can be drawn from the comparatively small number, is in agreement with the results of Smith (1932) and Messer. That the milk of large herds should more frequently be infected than that of small ones is what might be expected simply from the fact that they contain a larger number of individuals and are therefore not only more likely to contain an infected animal but more animals are exposed to any infection that may occur. The apparent incidence of infection as shown by such figures may therefore be a real one. On the other hand, in the examination of milk in Scotland for the tubercle bacillus (M.R.C. Report on Tuberculous Infection in Milk No. 189) it was found that the larger the quantity of milk from which the composite samples were taken the greater the proportion of infected samples. It is therefore possible that if the quantity of milk examined from each small herd was comparable to that produced daily by the larger herds a greater number of the small herds might be found to be infected.

The published records of the examination of milk for Brucella show that the number of infected samples found will not depend entirely on the number actually containing living Brucella but to some extent also on the procedure adopted in the examination, *e.g.*, size of the inoculum (quantity of milk centrifuged and whether cream as well as deposit is used), number of guinea-pigs inoculated with each sample whether only the sera of these animals are examined, or cultures only made, or both, and it may be added the tissues from which cultures are made. Beattie considered that an examination of the sera of the guinea-pig for agglutinins would alone give a very fair, though possibly not quite complete representation of the number of infected samples generally; and subsequent work has shown that this is on the whole true. On the other hand there are occasions, as in tracing the source of a human infection, when it is important to discover with as much certainty as possible whether a particular supply is infected and in such cases the examination should be as complete

as possible. Weighing all the evidence Pullinger considered the most suitable procedure to be the inoculation of each of two guinea-pigs with the deposit of 100 c.c. of milk *plus* 1 c.c. of gravity cream and the examination of the sera and spleens of the animals at the end of six weeks. The writer has found that an additional number of infected samples will be found if the local glands as well as the spleens of the animals are cultured. Van den Hoeden, however, has published some results showing that after subcutaneous inoculation of infected material from a variety of sources 85 of 92 (92 per cent.) spleens examined, gave positive cultures, whereas only 23 of 30 (76·7 per cent.) gland cultures were positive. He has not, however, correlated the results of gland and spleen cultures in the same animal. In the course of his work Pullinger found that particularly in the case of ordinary milk samples which may be heavily contaminated with extraneous organisms an examination of the milk whey for Brucella agglutinins might be the only evidence of infection in the herd. Though this could not be regarded as proof that the milk contained living Brucella it would justify further examination of the herd should the animals inoculated with the milk give negative results. Though six weeks may be the period of choice at which to kill the guinea-pig, Pullinger and Sheather found that evidence of infection might be obtained as early as the second and third week by examination of the serum for agglutinins. Pullinger found no additional positives in nine animals after three weeks, Sheather after six and Pröscholdt found that though 35 per cent. of guinea-pigs negative in a dilution of 1 : 20 in four weeks became positive in eight, only 3-4 per cent. negative in six weeks were positive in eight.

In view of the general experience that the majority of bovine strains of *Brucella abortus* isolated in this country require CO_2 for primary culture the comparatively large proportion of non-CO_2 sensitive strains found in the writer's series is of interest. This character is not recorded by all the workers who have isolated Brucella from milk, but Smith (1932) found two of 123 strains were not CO_2 sensitive, the same author (1934) found five of 33 strains not sensitive and Minett (1933) records non-CO_2 sensitive strains in seven of 22 cows in one herd. Though Beattie found all his 36 strains required CO_2 in early subcultures it would appear that non-sensitive strains are more common than was at one time supposed. Their significance is at present *sub-judice*, but in the present state of knowledge there are three possible categories to which they might belong if only ability to grow in ordinary air is considered. They might be porcine or even melitensis strains, or typical bovine strains such as are used in living vaccines which through culture in the laboratory have acquired the power of growing in ordinary air; or such strains may occur naturally in this country in a small proportion of cases being

similar to those regularly found in S. Rhodesia. It is scarcely necessary to say that apart from individuals who have been resident abroad, *Br. melitensis* is not known to occur in this country and though it is recognised that in America and some European countries porcine strains may infect cattle they too are unknown in this country under natural conditions; and even subclinical infections in pigs with Brucella of any type would appear to be rare, as has been shown by Doyle and Priestley who between them examined the sera of nearly 11,000 animals and found agglutinins in a 1 : 100 dilution in only four and in lower dilutions in only 22 animals, 0·23 per cent. in all. Unfortunately neither Smith nor the writer examined the non-CO_2 sensitive strains found by them by any of the other recognised tests for typing Brucella and it is thus impossible to say to what type they really belong. Several of the strains isolated by Smith were found in herds in which living vaccines had been used and he regarded them as vaccine strains which had settled in the udder. A living vaccine had also been used in the herd from which Minett's strains came. These were completely typed and found to be bovine strains but complete proof of their origin from vaccines is lacking as there is no record of the type of strain infecting the herd in which abortion had been occurring before the use of the vaccine. In the case of the writer's strains it was not possible to obtain information as to the use of a vaccine in any of the herds; but it is noteworthy that in three of the four ordinary milk farms from which non-CO_2 sensitive strains were isolated, CO_2 sensitive strains were also found; and it may be of significance that in the farm from which samples were received on three occasions, only a sensitive strain was found in the first, whereas in samples sent two and four months later both types occurred. Smith also found both types in the same herd. On the other hand, Professor Wilson kindly examined very completely a non-CO_2 sensitive strain isolated at the Institute early this year from a sample of Grade "A" T.T. milk and found it to be typically bovine. The owner of the herd, who was keenly interested, stated that though abortion had occurred in his herd two years before there had been none since and no vaccine of any kind had ever been used. This suggests that such strains may occur naturally. That the non-sensitive as well as the sensitive strains are of clinical importance is shown by the fact that they have been recovered from human infections in this country on three occasions (Wilson, 1932; Dalrymple-Champneys, 1933) and all of these, apart from their non-CO_2 sensitiveness, were typically bovine. As there is evidence that in America porcine strains cause a more severe illness in man than bovine strains it is important that all non-CO_2 sensitive strains found in this country from whatever host should be completely typed by their sensitivity to certain dyes, their serological reactions with mono-specific sera and other tests if necessary.

Summary.

One hundred and fifty-seven milk samples, the majority of which were ordinary milk, were examined for *Br. abortus* by guinea-pig inoculation. Thirty-four of these were found to be infected. An additional infected sample was found among 16 animals that died prematurely. *Br. abortus* was not isolated from the five pasteurised samples examined. The only sample of certified milk examined contained *Br. abortus*.

In more than half the samples cultures were made from the local glands as well as from the spleen. In six of 29 animals in this group from which positive cultures were obtained the organism was found in the glands but not in the spleen.

The sera of 42 guinea-pigs were examined for agglutinins and close agreement found with the cultural results. The only discrepancy was in favour of the cultural examination, but other series of animals somewhat similarly examined have shown that the reverse may occur.

Nine of 36 strains of Brucella isolated did not require CO_2 for primary culture or early subculture.

The choice of procedure in such examinations and the significance of the non-CO_2 sensitive strains is discussed.

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THE INFLUENCE OF GRAZING ON THE NATURAL IMMUNITY REACTIONS AND INTRADERMAL TOXIN TESTS IN SHEEP.

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The literature on the influence of nutrition on the natural immunity reactions has been reviewed in a previous paper by two of the present authors (1934) and more recently by Clausen (1934). This communication records the effect of grazing on these reactions in sheep.

The possibility of such an effect first appeared during the course of a feeding experiment on sheep carried out at the Rowett Institute in 1930 (see Orr, Macleod and Mackie, 1931). Several groups of sheep were fed on different diets, the groups being further subdivided so that half of the sheep were penned indoors and the other half outdoors in concrete pens. During the course of the experiment the animals most adversely affected were put to grass to recuperate. When the experiment was completed blood samples were taken from these animals after three months' grazing and certain natural immunological properties of their sera were compared quantitatively with those of the sheep which had been retained on the experimental diets until the end of the experiment.

The serological properties selected for the comparative tests were the following :—

(1) Hæmolytic reaction with rabbit erythrocytes.
(2) Bactericidal reaction with *B. suipestifer.*
(3) Bactericidal reaction with strains of *B. coli.*
(4) Agglutination reaction with *B. paratyphosus* B.
(5) Agglutination reaction with *B. abortus* (Hog).

The different mechanisms concerned in these reactions were referred to in the authors' paper cited above, in which the techniques used were also described. The same technique was used in tests (2) and (3), and also in tests (4) and (5).

While no significant differences appeared between the animals on the experimental diets in pens inside and outside, it was found that the average results obtained from all these animals showed a lower level of bactericidal activity to the organisms selected and also of hæmolytic power towards rabbit erythrocytes than did the grazing sheep. On the other hand, in the grazing sheep there was actually a lower level of agglutinating activity towards *B. paratyphosus* B and *B. abortus* (Fig. 1). Analogous reverse

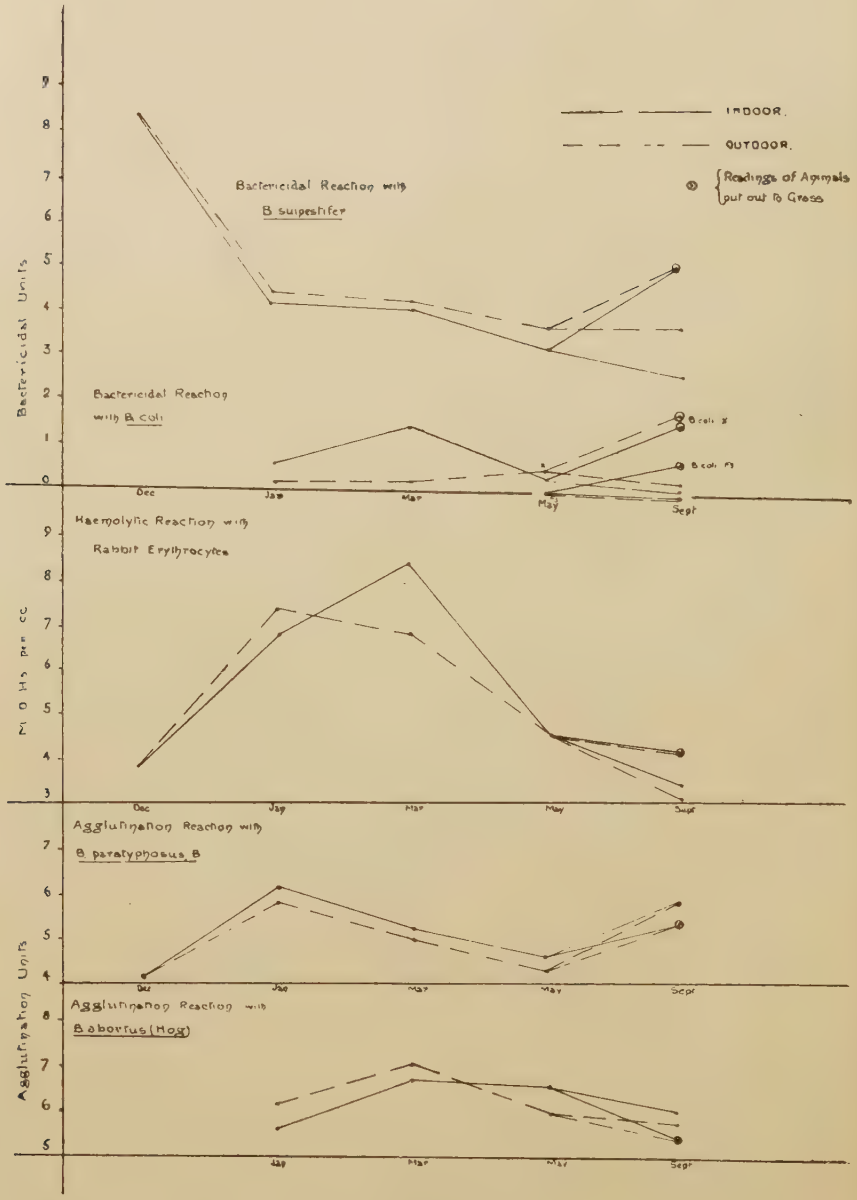

FIG. 1.

effects on lytic and agglutinating reactions by caloric deficiency have also been recorded by Anderson and Fraser (1934). Since the animals penned indoors showed no appreciable differences it seems that the effect of grazing cannot be attributed to sunlight or fresh air. The question still remains, however, as to whether such an effect is due to the nutritional effect of grass or to some

other effect of grazing such as exercise, natural grazing as opposed to bulk feeding, etc.

In a further experiment more evidence of the effect of grazing presented itself. During observations on a hill farm it was found that differences in natural immunity reactions in sheep which appeared to be correlated with supplementary feeding, and which were present in March, disappeared in early summer, as soon as young grass became available for all animals. This was especially noticeable in intradermal tests with lamb dysentery toxin (see Mackie *et al.*, 1932), while among the serological tests a similar significant result was observed in the hæmolytic reaction with rabbit erythrocytes.

The following experiment was now planned in an attempt to confirm the effect of grazing on natural immunity reactions and also to find out whether such an effect is due to the nutritional value of the grass or to some other condition associated with grazing.

Forty sheep which had been on a feeding experiment for some months and were in poor condition were arranged in three groups.

Group I was maintained indoors on the most complete of the experimental diets which had been fed previously. This consisted of :—

				Grams.	
Chaffed hay	50 parts		$CaHPO_4$	11	
			$CaCO_3$	11	
Chaffed straw	50 parts	Fed *ad lib.*	NaCl	55	56 grams
		+	Ground maize	22	daily
Polished rice	20 parts	Water *ad lib.*	Alfalfa meal	55	
			Yeast	1	
Blood meal	5 parts				

Cod liver oil 2·5 c.c. daily

Group II was kept in pens indoors and fed cut grass, or when that was not available, tares or rape.

Group III was turned out to graze.

The following qualitative tests were selected for estimation of natural immunity reactions :—

(1) Hæmolytic reaction with rabbit erythrocytes. (Due to a natural antibody acting along with complement.)

(2) Titration of complement.

(3) Bactericidal reaction with *B. coli* "X." (Due to a natural antibody acting along with complement.)

(4) Bactericidal reaction with *Streptococcus hæmolyticus*. (Due to the so-called β-lysin of serum.)

(5) Agglutination reaction with *B. abortus* (Hog). (Due to natural agglutinin.)

At the commencement of the experiment in June there was little difference in the average values for the three groups in each reaction. Sampling was carried out in July, September and on two dates in October. The results averaged in groups are presented graphically in Fig. 2.

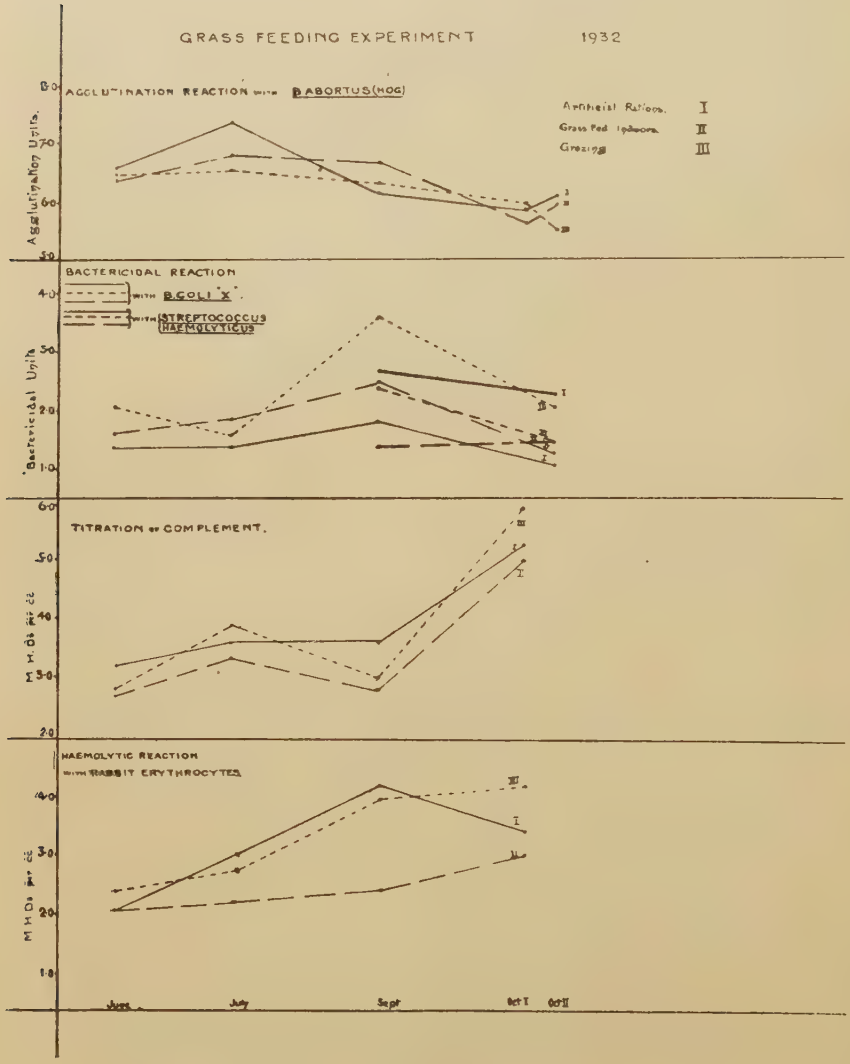

FIG. 2.

In June the average figures for Group III showed a slightly higher hæmolytic activity than Groups I and II. By July the hæmolytic titre in Group I had risen till this group showed a higher level of activity than Groups II or III, which had changed little. A further rise in Group I in September had a parallel in Group III, while Group II still showed little change from the value obtained in June. In October Group III was still rising, Group II showed a definite increase, while in Group I there was a diminution in activity. Thus at the end of the experiment the order of the hæmolytic activity of the sera of the various groups was III > I > II. Statistically the difference between Group III and either Group I or II was significant, while that between Groups I and II was not significant.

Throughout the experiment the complementing power in Group II was lower than in the other groups. Groups I and III alternated in position at the various samplings, Group III being highest at the end of the experiment, though not significantly so.

In the bactericidal reaction with *B. coli,* Group I gave the lowest readings throughout. In June Group III was somewhat higher than Group II but it lost this advantage in July. By September, however, it gave considerably higher readings than either of the other groups and this difference was still noticeable in October, though to a less extent.

The bactericidal test with *Streptococcus hæmolyticus* was carried out only in September and October, and on each of these occasions Group I gave the highest figure and Group II the lowest. In September Group III gave an intermediate value, but in October it was identical with Group II. It should be noted that the bactericidal effect of animal sera towards *Streptococcus hæmolyticus* is due to a different mechanism than the similar reaction with *B. coli* (Mackie and Finkelstein, 1932) (*vide supra*).

The differences between the groups in the agglutination reaction with *B. abortus* (Hog) were very small at all times and the relative positions of the groups varied at each sampling. It is interesting to note, however, that at the end of the experiment Group III yielded the lowest average.

At the end of the experiment intradermal tests were carried out with lamb dysentery toxin. Two inoculations were made into the skin at the base of the tail, the doses used being 0·5 c.c. of 1 in 15,000 and 1 in 7,500 dilutions. These doses had been found previously to lie on the borderline as regards inducing any skin reaction or not. With the 1 in 15,000 dilution 8 out of 14 sheep (56·5 per cent.) in Group III gave no reaction, while in Groups I and II respectively the proportion of negatives was 2 out of 12 (16·5 per cent.) and 4 out of 11 (36·2 per cent.). (Fig. 3.) This agrees with the results found in the hæmolytic reaction.

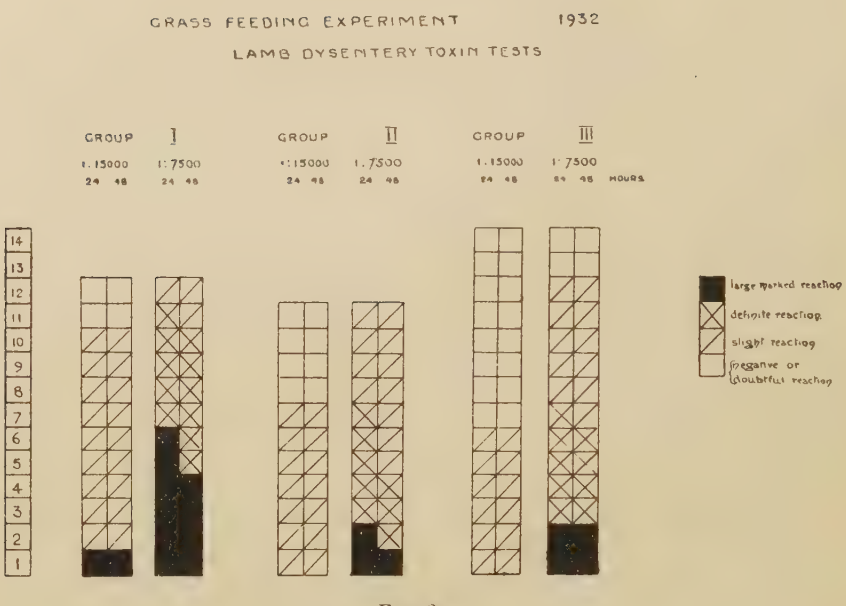

FIG 3.

CLINICAL OBSERVATIONS.

At the outset of the experiment all sheep were in rather poor condition. When they were arranged into the three groups this was done so that those in best condition were maintained on the artificial ration and the poorest animals were put to graze. After two months (*i.e.*, in September) both Groups I and III showed a marked improvement, but Group II did not. At the end of the experiment the sheep in Group III were healthy and had improved greatly in condition and those in Group I were fairly healthy, but the rate of growth during the later stages was not so rapid as in Group III, and at the last weighing a fall in weight was observed. On the other hand, the animals in Group II, though getting all the grass or rape they could consume, did not thrive well, but at the last weighing they did show an increase in weight. Unfortunately it was not possible to carry the experiment further in order to see if this rise was maintained as no further supply of green foodstuff was available at this time of year. During the course of the experiment the average increase in weight in Groups I, II and III respectively was 15 lb., 8 lb. and 22 lb.

DISCUSSION.

The experiment was planned, as mentioned above, to confirm observations made regarding the effect of grazing on the natural immunity mechanism and to find out, if possible, whether such an effect were due to the nutritive properties of the grass itself or to some other factor associated with the conditions of grazing.

Such factors might include sunlight and fresh air, exercise, grazing throughout the day, as opposed to bulk feeding, etc. The conditions of the experiments in which the effects of fresh grass first appeared seem to discount the influence of sunlight and fresh air. In order to make the two groups which were receiving grass in this experiment strictly comparable, the grass fed to the animals indoors should have been cut from the field in which the other group were grazing. Unfortunately, not only was this impossible, but also there was an insufficient supply of cut grass available for the whole duration of the experiment and during some periods rape and tares had to be fed. It was noticed that on some occasions even newly-mown grass was rejected by the animals and it would appear therefore that they were not receiving that part of the pasture which they would select if they were grazing. It is thus difficult to say whether the differences observed between the group grazing and that fed cut grass is due to the food itself or to some other factor. Grass, when obtained by grazing, leads to differences in the natural immunity reactions as compared with an artificial cereal diet. Cut grass, however, when fed in bulk, produces no differences or, at most, slight differences.

Summary.

(1) The natural hæmolytic activity of sheep serum towards rabbit erythrocytes was found to be higher in grazing animals than in comparable sheep on an artificial ration.

(2) A similar difference was noted in the natural bactericidal reaction towards a strain of *B. coli*.

(3) No such difference was apparent in the complementary power of the serum or in the β-lysin (tested with *Streptococcus hæmolyticus*) or agglutination reaction with *B. abortus*.

(4) A group of sheep fed on fresh green food in indoor pens did not show the same increase in the two reactions mentioned above as did the grazing group.

(5) The proportion of sheep showing a negative reaction to a given dose of the toxin of the lamb dysentery bacillus (*B. welchii* type B) was greater in the grazing group than in those fed on green food and on the artificial ration.

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SOME EXPERIENCES WITH POTASSIUM ANTIMONYL TARTRATE IN THE TREATMENT OF BOVINE TRYPANOSOMIASIS IN NIGERIA.

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Potassium antimonyl tartrate is still generally regarded as the sheet anchor in treating animal trypanosomiasis. Various new compounds have appeared from time to time and have been used either alone or in conjunction with tartar emetic with varying success. On the whole the results obtained have not been so striking as to justify their adoption on any scale. Expense of treatment is usually a ruling factor. As tartar emetic is relatively cheap, and as its use has been followed by reasonable success in the hands of the majority of workers in this field, it will be difficult to displace. An indirect advantage is the fact that trypanosomes do not readily become fast by the ordinary direct method to this drug (Yorke, 1932). This advantage should not be disregarded, since complete and sterilising courses of treatment are seldom practicable or given in the field. This article records a few of the results observed when using tartar emetic as the trypanocidal agent in bovine trypanosomiasis in Nigeria. It was owing to an unexpected result when using the drug in 1931 that these experiments with tartar emetic, utilising various Nigerian strains and combinations of strains, were carried out.

The 1931 experiment referred to above was intended to throw some light on the state of premunition set up in infected cattle when treated with tartar emetic. Thus we wished to show how long this premunition would last and if it could be maintained by repeated minimal infections through laboratory-bred infected Glossinæ. The experiment was not completed owing to the high mortality during and just after treatment with tartar emetic, details of which will be given later.

All the cattle used in the following experiments were of the humped type purchased locally and varied in age from 18 months to five years. They were all entire males, and when arranged in groups for experiment the age distribution was as far as possible similar. They were examined by means of blood and gland smear (wet and stained) for some time before being used and any showing trypanosomes were discarded. There is, however, no guarantee that any or all of the cattle had not at some time a mild trypanosomal infection and recovered, but at the time of use the blood was devoid of parasites and the animals were, with one group exception, in good condition and doing well.

The strains of the trypanosomes were all recently isolated from wild *G. morsitans* and had undergone at the most one or two syringe passages.

The tartar emetic was always made up freshly in bulk as a 4 per cent. solution in saline and sometimes in distilled water. The doses varied at the outset and are indicated, but finally 0·06 to 0·08 c.c. of the 4 per cent. solution in saline per kilo. body weight was taken as the standard. The smaller dose was given to weak and debilitated animals and is considered essential in those cases. The maximum dose given was 24 c.c. of a 4 per cent. solution, to animals weighing 300 kilos. or over. The standard interval between doses was six to seven days and the total quantity of drug given varied from 3 to 6 grammes.

(1) *Treatment of Double Infection* (T. vivax *and* T. congolense) (*Syringe Passage*).

This experiment, to which reference has already been made, was primarily designed to study the state of premunition in trypanosomiasis. It is recorded here as a tartar emetic treatment experiment. Forty cattle were inoculated simultaneously with strains of *T. vivax* and *T. congolense* on February 23rd, 1931.

The strains were isolated in December, 1930, from wild *G. morsitans* by feeding on a sheep. *T. congolense* was obtained pure by one syringe passage through the dog, from which it was passed to a clean sheep. The *T. vivax* strain had two syringe passages in sheep. The dose given to the cattle was approximately half a million trypanosomes of each strain, estimated by trypanosome count and suitable dilution. The incubation period judged by the first detected appearance of trypanosomes in the blood varied from eight to 17 days. The disease was allowed to run its course for a little over a month, and the blood was examined every second or third day. The usual intermittent course was shown by every animal, but the length of the negative and positive phases varied individually. Treatment with tartar emetic was begun on April 8th, 30 animals being given the drug and ten controls not treated. The dose given was 25 c.c. of a 4 per cent. solution in distilled water as recommended by Richardson (1928). There was no weigh-bridge available at this time, but approximately the weights varied from 250-350 kilos. On the day following this injection two animals died, two days after three died and three days after one died. The absence of such mortality among the uninoculated controls threw suspicion on the tartar emetic solution. The water definitely was quite pure and the drug itself was lead-free for intravenous injection as supplied by British Drug Houses, Ltd. Cawston (1929) suggested that the system might react unfavourably to the injection of much distilled water into the venous circulation, so solutions were subsequently made up in normal saline. While later work, in which

injections were controlled by the Van den Bergh test, convinced us that accurate dosage was of primary importance when using tartar emetic in cattle, the precautionary use of saline should not be overlooked. Curson (1922) recommends 1·5 grammes of tartar emetic for adult cattle, but after our experience noted here we would hesitate to give anything like so much to adult Nigerian cattle infected with trypanosomes.

On the day following the second injection, 25 c.c. of 4 per cent. solution in saline, given on April 15th, two deaths occurred. Subsequently $\frac{1}{2}$ gramme doses were given or 12·5 c.c. of the solution at weekly intervals until a total of 4 grammes had been given. No more deaths occurred during treatment. Observations were continued for a further five months after treatment was completed. Relapses during this time were noted in all but two of the treated animals, in one animal as early as ten days after the last injection of tartar emetic. Sporadic deaths occurred in the treated group as follows : one in June, two in July, two in August, three in September and three in October. Deaths did not occur among the controls until July, four months after they were infected—two died in July, one in August, one in September and two in October. Thus at the end of the experiment, about six months after treatment began, there were eleven survivors out of 30 in the treated group and four out of ten in the untreated.

(2) *Treatment of Double Infection* (T. vivax *and* T. congolense) (*After Recovery from Rinderpest*).

A group of 60 bulls, ages ranging from two to four years, were inoculated on January 11th, 1932, with half a million trypanosomes each of *T. vivax* and *T. congolense*, the strains having been recently isolated from wild *G. morsitans*. For the purpose of observing the effect of feeding minerals on the course of the disease they were divided into six groups of ten animals. From the day after inoculation and thereafter every alternate day five groups of animals were given different minerals by the mouth and the sixth or control group received nothing at all. The average initial weights of the animals in the various groups was 230 kilogrammes and the average red cell count taken before infection was in the region of 6×10^6 per c.c. Only a very limited number of animals showed a count of 8×10^6 or over, the accepted normal blood count of cattle in South Africa. The disease ran a normal course for three weeks with the usual rise and fall of trypanosomes in the blood. There was a comparatively rapid fall in weight and in the number of red cells. The existence of rinderpest in a number of animals was confirmed on February 15th. A good number of the animals had been artificially immunised against rinderpest and the remainder were given serum alone as soon as possible. Forty-six animals survived the outbreak.

It was decided that these 46 animals should be treated with

tartar emetic and observed over a reasonably long period of time. Treatment was begun on March 18th, over two months since they were artificially infected with *T. vivax* and *T. congolense*. They were in poor condition and the red cell counts were 40-50 per cent. below normal. The dose of tartar emetic given was 0·08 c.c. of a 4 per cent. solution per kilo. in saline and the total quantity of drug administered was a little over 6 grammes. Only four animals died during the course of treatment. The animals were kept under observation for a further period of six months and during this period relapses were frequent and sporadic deaths occurred. During the first three months of this period eight animals died, during the second three months the mortality was much higher, 22 dying. *Post-mortem* examinations were carried out in all but seven cases and death was attributed to trypanosomiasis in all those that it was possible to examine. The total mortality, including the four deaths during treatment, was 30 out of 46 animals, or 74 per cent. In spite of the fact that there were no untreated controls in this group, the result does show that treatment was not very successful no matter what lack of treatment might have shown.

(3) *Treatment of Double Infection* (T. vivax *and* T. congolense) *with Maximum and Graduated Doses.*

Edwards (1926) found that in horses, after giving a full therapeutic dose intravenously, tartar emetic remains in trypanocidal concentration in the blood stream for 24 hours only. Therefore he repeats treatment on alternate days. He states, too, that when he gave a full therapeutic dose to horses with an intense trypanosome septicæmia death often followed either from occlusion of the blood capillaries or from liberation of a toxic substance by the dead trypanosomes. He therefore advises treatment in the early stages with small doses. Cross and Patel (1922) in India, and Leckie (1925) in the Sudan, advise treating camels by beginning with small doses at short intervals, gradually working up to a full dose. In view of these reports it was decided to compare treatment of double infection bovine trypanosomiasis when full doses at weekly intervals were given and when small gradually-increasing doses at short intervals were given, the total quantity of drug being the same in each case. Thirty animals were used, ten treated by each method and ten untreated controls. All the animals were inoculated with approximately half a million *T. vivax* and half a million *T. congolense* on April 25th, 1932. Weights were taken monthly, red cell counts made weekly, and wet blood films examined every third day. The incubation period was normal but weights and red cell counts fell rapidly. When the latter counts were about 50 per cent. below normal treatment was begun.

Group I received maximum doses—0·08 c.c. of a 4 per cent. solution of tartar emetic in saline per kilo. body weight, at weekly intervals, until at least 4 grammes had been given.

Group II was treated as follows: The drug was given every second or third day, the initial dose being 0·03 c.c. of the 4 per cent. solution per kilo. This dose was increased at every injection by 0·01 c.c. of the solution per kilo. until the maximum 0·08 c.c. per kilo. was reached and the total quantity of 4 grammes given.

The infection in this experiment proved to be a virulent one, as the first control died on May 26th and the last of the ten died on July 13th, *i.e.*, two-and-a-half months after inoculation.

After the last control had died seven animals remained in each of the treatment groups. Two months later five animals remained in Group I and four animals in Group II. Within another month one died in Group I and two in Group II. Since the difference between the two groups is not significant it would appear that the extra labour involved in giving graduated doses over a longer period of time is not justifiable.

Considering the treatment with tartar emetic as a whole in this experiment, out of 20 animals six survived after six months as compared with a group of ten controls which all died within two-and-a-half months.

(4) *Treatment of* T. vivax *Infection* (*Direct* Stomoxys).

The treatment experiments already described refer to double infection with both trypanosomes. It is generally recognised and it has been our experience in Gadau that such infections are, as a rule, more virulent for cattle and are consequently more difficult to treat than single infections with either *T. vivax* or *T. congolense* alone. It was desirable to have some definite information about the effect of tartar emetic on such single infections.

A batch of about 170 young bulls were purchased in October, 1932, for different experiments. During the preliminary blood and gland examinations for elimination of any infected animals only three such animals were discovered. About a month later while such examinations were still in progress there was a sudden and alarming flare up of pure *T. vivax* infection among the cattle and in about five weeks 126 were shown to be infected. Tstetse fly were eliminated as vectors or being responsible in any way for this 74 per cent. infection. The bush had been cleared all round the camp for a distance of over a thousand yards and repeated fly surveys failed to reveal a single tsetse fly in the grazing areas and the density of the fly ten yards inside the fringing bush was only 1·5 per fly boy hour. Further, the cattle never grazed nearer than 500 yards to this uncleared bush. One or two isolated tsetse brought into the cleared area by donkeys or motor cars could not have caused the infection described. It may be said, then, that direct infection with *Stomoxys* and/or

other biting flies was the cause of this occurrence, as conditions were ideal for this to take place, *viz.*, the presence of a very virulent *vivax* strain in close proximity to the inported animals and relatively large numbers of cattle kept in close contact in paddocks morning and evening at a season of the year when biting flies were very numerous. Further, when the remaining cattle which had never shown trypanosomes in the blood were isolated the spread of the disease was finally stopped.

These cattle, having become infected with *T. vivax* in the manner described, were arranged in three groups for a tartar emetic treatment experiment as follows:—

Group I.—Twenty animals were treated as soon as possible after the infection with *T. vivax* was demonstrated. The total quantity of the drug given was 5 grammes in doses of 0·08 c.c. of a 4 per cent. solution of tartar emetic per kilogramme body weight given every six days.

Group II.—Eighty animals were set aside and the disease was allowed to run a natural course for at least two months. They then received a course of treatment with tartar emetic similar in every way to Group I.

Group III.—Twenty animals receiving no treatment and acting as controls for both of the above groups.

All the above animals were kept under observation for ten months after treatment was completed, the blood being examined every third day and the animals weighed monthly. *Post-mortem* examinations were carried out on all the animals dying with very few exceptions, where *post-mortem* changes precluded such examination.

In Group I 18 animals only received the full course of tartar emetic as one died after the first and one after the fourth injection of the drug. After a lapse of three months 17 animals were still alive as compared with twelve alive in the control group at the same time. During the following seven months, however, there were sporadic deaths following relapses and the survivors ten months after treatment numbered 13, a mortality of 35 per cent. Ten controls survived during the same length of time, a mortality of 50 per cent.

In Group II, of the 80 animals left untreated for two months and then given a similar course of tartar emetic 35 only survived the arbitrary period of ten months following treatment. As before, the control mortality was 50 per cent.

(5) *Treatment of Pure* T. vivax *Infection in Cattle (Cyclical).*

Twenty bulls were infected by feeding infected laboratory-bred *G. tachinoides* as follows: 800 laboratory-bred *G. tachinoides* were first of all fed on a bull showing *T. vivax* only in the blood, every alternate day for three weeks. Subsequently 245 of these flies were fed on each of the 20 bulls on two separate occasions.

Dissection of the flies after the feeding was completed showed a pure *T. vivax* infection.

One animal had to be destroyed, and in two trypanosomes could not be demonstrated, leaving 17 for the experiment. Eight were treated with tartar emetic—0·08 c.c. of 4 per cent. solution per kilo., a total of 5 grammes being given. Nine animals were untreated as controls. The animals were kept under observation for a period of ten months after treatment was completed and during this period five animals died in the treated group and four in the untreated.

(6) *Treatment of Pure* T. congolense *Infection in Cattle (Cyclical).*

Three hundred laboratory-bred *G. tachinoides* were infected with *T. congolense* by feeding on a dog. This strain of *T. congolense* was isolated in December, 1932, from wild *G. morsitans* in a dog and passed on by syringe passage. One hundred and twenty-two of these infected flies were fed on each of 20 bulls on four occasions at intervals of ten days. The appearance of trypanosomes was very irregular and the infection usually scanty—one parasite in every five or six fields, 1/6 objective. In three animals trypanosomes could not be demonstrated at all. In spite of this fact the strain proved to be quite virulent. Only the 17 animals in which trypanosomes were demonstrated were utilised, six being treated with tartar emetic 0·08 c.c. of a 4 per cent. solution per kilo. and eleven were not treated. After a lapse of six months after treatment all the untreated controls had died, while four of the six receiving treatment were alive, a mortality of 33 per cent.

(7) *Treatment of Double Infection* (T. vivax *and* T. congolense) *(Cyclical).*

In the earlier experiments the animals treated for the double infection disease were infected by means of the syringe. For comparison a group of 20 animals were infected with both trypanosomes by means of laboratory-bred infected fly. The strains were obtained from wild *G. morsitans* in a sheep. Three hundred laboratory-bred *G. tachinoides* were fed on this sheep as soon as *T. vivax* and *T. congolense* could both be demonstrated. Subsequently batches of 115 infected flies were fed on each of the 20 experimental cattle utilised, on two occasions at an interval of ten days. The infection set was very virulent indeed and those infected early had succumbed before the treatment could be started as an experiment. It was found impossible to have a sufficient number of infected flies available at one time to feed on all the cattle simultaneously. Seven of the 20 animals could not be considered. Of the remaining 13, six were treated as before, 0·08 c.c. of 4 per cent. tartar emetic and 5 grammes given, and seven acted as controls. Eight months after treatment all the

controls were dead, while three of those treated were still alive. Table I gives details of this experiment.

TABLE I.

T. congolense AND *T. vivax* CYCLICAL INFECTION WITH LABORATORY-BRED *G. tachinoides*.

No.	*Dates 1st Fly Feeding.*	*Incubation Period Trypanosomes first seen in Blood.*	*Completion of Treatment.*	*Days before Relapse.*	*Wegiht in Kilos. 18/1/33.*	*Date of Deaths.*	*Weight before Death or at end of Experiment.*
		Days					
52	2.2.33	10	23.3.33	25	225	13.5.33	199
76	29.1.33	14	„	12	229		293
82	4.2.33	10	„	12	229	24.5.33	189
141	1.2.33	9	„	18	286		328
184	31.1.33	12	4 injections	Died before treatment completed	342	9.3.33	331
191	31.1.33	10	23.3.33	128	318		320
9	5.2.33	7			169	27.3.33	164
18	2.2.33	14	Con-		219	17.3.33	215
54	29.1.33	12	trols		178	14.4.33	169
168	30.1.33	11	no		172	25.3.33	170
182	3.2.33	13	Treat-		316	28.3.33	298
192	4.2.33	8	ment		238	26.4.33	222
206	5.2.33	11			197	20.3.33	195

Table II summarises the findings of the various experiments.

TABLE II.

Type of Infection.	*Date.*	*Number of Animals.*	*Treatment.*	*Months between End of Treatment and Close of Experiment.*	*Number Alive.*	*Per cent. Mortality.*
1. Double infection of *T. vivax* and *T. congolense* given by syringe	1931	30	2 doses 1 grm., 3 doses ½ grm.	5	11	63
		10	Nil.		4	60
2. As above. The animals were in poor condition after recovery from rinderpest	1932	46	Dose according to weight 0·08 c.c. of 4 per cent. T.E. per kilo. Total of 6 grms. given	6	12	74
3. Double infection of *T. vivax* and *T. congolense* given by syringe	1932	20	10 given full dose, total 4 grms. 10 given graduated doses, total 4 grms.	6	6	70
		10	Nil.	(2½)	0	100
4. *T. vivax.* Naturally acquired direct infection (*Stomoxys*)	1933	80	Treated with full doses after disease ran its course for 2 months.	10	35	56
		20	Treated early within 1 month	11	13	28
		20	Nil.	(12)	10	50

TABLE II (*continued*).

Type of Infection.	*Date.*	*Number of Animals.*	*Treatment.*	*Months between End of Treatment and Close of Experiment.*	*Number Alive,*	*Per Cent. Mortality.*
5. *T. vivax* (Cyclical infection)	1933	8	0·08 c.c. 4 per cent. T.E. per kilo., total 5 grms.	10	3	62
			Nil.	(10)	6	44
6. *T. congolense* (Cyclical infection)	1933	6	0·08 c.c. 4 per cent. T.E. per kilo., total 5 grms.	6	4	33
		11	Nil.	6	0	100
7. Double infection (Cyclical) *T. vivax* and *T. congolense*	1933	6	0·08 c.c. 4 per cent T.E. per kilo., total 5 grms.	8	3	50
		7	Nil.	8	0	100

Discussion.

Considered as a whole these results are not very impressive. The question of the effect of treatment with tartar emetic in this disease is one which has given rise to a great deal of argument. To anyone who has had the opportunity of using the drug and observing its effects on a reasonable number of infected cattle it is not surprising that such difference of opinion should exist. One observer called upon to treat a group of cattle infected with a particular species of trypanosome might, when using tartar emetic, be perfectly satisfied with the apparently good results achieved. Following the temporary sterilisation of the circulation there is often a quite visible improvement in condition and a negligible mortality. Observations are rarely continued beyond two months, or at the most three. On the other hand, another observer might, when using the same treatment technique against a different strain of the same species, be equally dissatisfied. It is most unwise to be dogmatic either way with regard to tartar emetic, as the results achieved depend entirely on two variables, *viz.*, the susceptibility of the animals and, more important, the virulence of the parasite or parasites. The variation in virulence between species and also between strains of the same species recently isolated from wild fly is at most remarkable. In one test carried out a strain of *T. vivax* killed ten cattle within a period of three months, while within the same period of time a strain of *T. congolense* failed to kill a single animal out of ten. A similar test carried out at a later date showed that the mortality produced by each species over a fixed period of time to be about equal. Observations over a period of five years at Gadau have constantly shown *T. vivax* to be the commoner trypanosome of the two in this area of Nigeria, though reports from other parts of the country state that *T. congolense* is of greater economic importance. This question apart there can be no doubt that in *G. morsitans*

areas at least the danger from infection with both trypanosomes at one and the same time is very real, and without exception we have always found this type of the disease to be uniformly virulent and most refractory to drug treatment. The problem in Nigeria is somewhat different. The number of working oxen is infinitesimal at present and the majority of cattle are owned by nomad Fulani. These cattle owners bring their cattle to various veterinary camps chiefly for rinderpest inoculations and during the quarantine period trypanosomiasis treatment is carried out. As soon as the quarantine is removed they must move off to new grazing areas, and so long courses of treatment are not practicable. An effort has been made to ascertain the real value of giving a few injections of tartar emetic, by keeping animals under observation for a reasonably long time. Temporary improvement alone might enable the cattle owner to sell off a few surplus males to the butcher but females he will not sell as they represent his capital. The premunition set up by using tartar emetic was in some cases little better than that in the controls, where no drug was used. If the disease is treated in the early stages the effect is more marked, the tartar emetic apparently checking the early mortality which occurs among less resistant animals. In acute infections tartar emetic appears to exercise a slight benefit only.

Although it is probable that the use of tartar emetic may help animals to combat the very mild infections met with in the field, we are of the opinion that some other drug is urgently required if we are going to cope with bovine trypanosomiasis and make real progress under present conditions in Nigeria.

We have to acknowledge with thanks the assistance of M. H. Julde and P. S. Dawodu in carrying out the large number of routine examinations.

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A SPECIES OF DEMODEX FOUND IN SHEEP IN BRITAIN.

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Records of *Demodex* infection of the sheep are of rare occurrence in the literature and, although Hirst (1919, 1922) considers that such mites are probably common parasites of this animal, he records their presence only in the Meibomian glands of one recently slaughtered sheep. This appears to be the only record of *Demodex* infection in sheep in this country. There are a few records of the parasite causing a type of mange in sheep on the Continent and in Africa, and it is also believed to occur in the United States and in India, the shoulder, flanks and neck being the areas usually attacked. Griffiths (1915) describes the disease thus: "Slight pustular eruptions which gradually coalesce are usual, while later there is thickening of the skin and loss of wool. Emaciation is very marked and many animals die."

The work described in this paper originated through the finding of certain organised bodies in a section of skin from the region of the vulva of a ewe affected with scrapie, a disease of sheep in which intense itch is a prominent symptom. The nature of these bodies was at first obscure, because of the effect of their being sectioned in different planes. The bodies were present in the sebaceous glands of the skin adjoining the mucous membrane of the vulvar commissures. They were identified by Dr. Russell Greig, Director of the Institute, as a species of *Demodex*, an observation confirmed by Dr. A. E. Cameron of the Zoology Department of the University of Edinburgh.

In view of these findings, it was resolved to examine a number of scrapie sheep for the presence of *Demodex*. The examinations were at first specially directed to the region of the vulva and to those other areas of skin in which the sebaceous glands are large and numerous. Such areas exist on the perineum, on the inner aspect of the thighs where the skin is devoid of wool, on the prepuce, around the nostril and mouth and inside the eyelid (Meibomian glands).

Preparations from the skin of healthy sheep and of sheep suffering from conditions other than scrapie were also examined.

Technique.

The method generally adopted for the examination of skin consisted in fixing the material in 4 per cent. formol, cutting frozen sections and staining with hæmatoxylin and eosin.

Isolated specimens of the parasite were obtained by exerting

sustained, moderate pressure on a fold of affected skin. By this means comedones are pressed out of the sebaceous glands. The region of the preputial orifice, which contains large sebaceous glands, has been found the most satisfactory area from which to obtain specimens. The comedones were teased out in Berlese's fluid on a slide, mounted in this fluid and examined. During the process of clearing, considerable distortion is liable to occur before the mite is fully cleared. The measurements given in Table II were made before distortion had occurred, except the head measurements, which were made after the preparation was cleared.

Section of skin of vulvar region from a sheep affected with scrapie. x 135

A. Mites lying in sebaceous glands.
B. An unaffected sebaceous gland.

Experimental Results.

In nine of the twelve cases of scrapie examined *Demodex* was found in the skin around the vulva. The affected cases showed considerable variation in their degree of infestation. The accompanying illustration shows a section of skin in which the infestation was a relatively heavy one.

Examination of skin preparations obtained from the other regions mentioned above—in one case from the peri-anal region, in seven cases from the inner aspect of the thigh, in ten cases from the region of the nostril, in one case from the lips, and in nine cases from the eyelids—revealed the presence of a few

Demodex in the skin of the nostril of two sheep (the vulvar region was also infected in both these cases). Attention was then turned to an examination of those areas of skin which showed the most marked evidence of pruritus—the back, the sides and the hind-quarters. Sections from nine such areas obtained from seven cases of scrapie were examined for the presence of the mites, with consistently negative results.

Previous workers on scrapie have not recorded the finding of acari or other parasites in the skin.

As a control to these observations, skin preparations obtained from a number of sheep free from scrapie were examined. The results are shown in Table I.

TABLE I.

Description of Sheep.	*Material Examined.*	*Presence of Demodex.*
13 healthy sheep varying in age from about 8 months to over 3 years old. (These animals were being slaughtered for food.)	Skin of vulva	All negative
2 ewes with Johne's disease (1 Half-bred 3 years old, and 1 Blackface 4 years old).	„	Both positive
2 Cheviot sheep, 2 years old, both emaciated	„	One positive and one negative
1 Half-bred ewe with abscesses on the face...	„	Positive
1 Blackface ewe with gangrenous rumen ...	„	Negative
1 Blackface ewe in good condition	„	„
2 aged Blackface ewes (destroyed for louping-ill vaccine preparation).	„	One positive and one negative
2 ewe hoggs (under 1 year old), 1 Blackface and 1 Half-bred, both emaciated.	„	Both negative
1 Half-bred wether hogg (used for louping-ill vaccine preparation).	Skin of Anus	Negative
1 Blackface aged tup (with septic pleurisy)...	„	„
1 aged Cheviot ewe (" chouger ")	Skin of vulva	Positive
1 Half-bred ewe hogg	„	„
4 aged Blackface tups (destroyed for louping-ill vaccine preparation).	Skin of prepuce	All positive
1 Blackface tup hogg (emaciated)	„	Negative

Hirst (*loc. cit.*) gives the measurements of the mites found by him in the Meibomian glands. These are shown in Table II along with similar measurements of the mites described in this paper. In addition to the mites described in the table, five mites, three of a total length of 254μ each, one of 273μ and one of 276μ in total length, were observed. The sex of these was not noted, and for this reason they are not included in the table of measurements.

Discussion.

Since the mites described in this paper were first found in the sebaceous glands of the skin in the region of the vulva of a case of scrapie, an examination of further cases of scrapie was carried out in order to determine whether the presence of the parasite was

of any etiological significance. The vulvar region was examined along with the other areas of skin rich in sebaceous glands, and those areas in which evidence of pruritus was most marked. The results showed that *Demodex* is a common, though not a constant parasite of the vulvar region of sheep affected with scrapie. In the examination of those parts other than the vulvar region, the only positive finding was an infection of the skin of the nostril in two cases, indicating that the parasite is not widespread over the skin in scrapie. Subsequent examination of skin preparations from healthy sheep and, also, from sheep suffering from disease conditions other than scrapie, revealed a number of cases of infection with *Demodex* (Table I).

The parasite does not therefore appear to be of etiological significance in scrapie since :—

(i) The parasite is not peculiar to sheep affected with scrapie.

(ii) It may be absent from sheep suffering from this disease.

(iii) Its presence was not demonstrated in those areas of skin which showed most marked evidence of pruritus.

The fact that *Demodex* was found in none of the 13 abattoir sheep examined suggests that sheep in good condition are relatively insusceptible to the infection. Conversely, of the other 18 control sheep in which the vulva or the prepuce was examined, the majority of which animals were diseased and in poor condition, 61 per cent. harboured the parasite, and 75 per cent. of the scrapie sheep examined (all of which were markedly debilitated) were found to be infected. From this evidence it appears reasonable to conclude that a condition of debility renders sheep more susceptible to infection by this parasite.

No specific inflammatory reaction was observed in the tissues in which the parasites were embedded, and there was no evidence of skin irritation in the affected area. It should, however, be recorded that in one case in which the prepuce was heavily infested by the parasites, erosion of a small area of skin occurred in the region of the preputial orifice; the cause of this erosion was not determined.

Many of the infected controls were under observation at this Institute for some time before they died or were destroyed, and in no case were symptoms observed which could be attributed to irritation from *Demodex* infestation. Two of the infected control sheep, suffering from diseased conditions erroneously diagnosed by their owner as scrapie, though particularly observed, did not show any symptoms of itch.

Though infestation by the species of *Demodex* described in this paper appears to be of fairly common occurrence, there is no record of such infestation producing a condition of clinical significance in sheep in this country. The *Demodex* recorded in sheep abroad may be of a different species, and one which has a

greater tendency to become widespread over the skin. On the other hand, the species may be the same as the one here described, while conditions may not be sufficiently favourable in this country to allow of its establishing itself in areas of the skin other than those in which conditions are particularly suitable, such as the perineum and prepuce.

An examination of Table II shows that the mite described in this paper is much larger than that found by Hirst, while the

TABLE II.

	Males.		*Females.*		*Hirst's Measurements of Females* (1919).
		Average.		*Average.*	
	μ	μ	μ	μ	μ
Total length of body (including capitulum)	301–414	372	286–409	342	221–233
Length of cephalothorax + capitulum	98–114	108	95–133	120	87–94
Length of abdomen	144–300	253	172–301	223	134–139
Greatest width of cephalothorax ...	51–67	58	51–76	64	44
Greatest width of abdomen ...	38–48	42	41–60	49	45
Greatest width of capitulum (at base)	27–34	30	25–35	30	29
Length of capitulum	17–24	20	17–24	20	22
Spines on capitulum	1·5–2·0 (about)		1·5–2·0 (about)		3 (about)

Measurements of the total length, of the cephalothorax and of the abdomen were made on 21 females and 9 males.

Head measurements were made on 11 females and 8 males and, in the case of the spine, on 9 females and 4 males.

spines on the capitulum are definitely shorter. The spines are, in fact, relatively inconspicuous objects.

It is, therefore, possible that the mite described in this paper represents a species distinct from that recorded by Hirst.

Conclusions.

(1) A species of *Demodex* is a fairly common parasite of sheep in this country. It is found most frequently in the sebaceous glands of the skin in the region of the vulva and the prepuce.

(2) There are indications that it is more common in debilitated and emaciated sheep than in sheep in good physical condition.

(3) The parasite appears to be benign and of no pathogenic significance.

Acknowledgments.

It is a pleasure to thank Dr. A. E. Cameron for help with the technique of mounting and measuring the mites and Mr. G. H. Annat for preparing the photograph.

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HYPOMAGNESÆMIA IN EQUINE TRANSIT TETANY.

H. H. Green, W. M. Allcroft and R. F. Montgomerie.

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About six years ago Montgomerie, Savage and Dodds (1929) described a tetanic disorder in Welsh mountain ponies (of both sexes) which had just come off a railway journey under cramped conditions, stating that its occasional occurrence had been recognised for over 20 years and correlating it with the disease described by Harvey (1923) in a moorland pony transferred from grazing to stable. The conditions of occurrence, symptoms and response to treatment are discussed in the paper of Montgomerie *et al* and need not be covered again, beyond recalling that the outstanding physiological features were fibrillation, clonic spasms, and increased respiration without marked elevation of temperature, while biochemical examination of the blood in two cases revealed hypocalcæmia and alkalosis.

In the interval the commoner bovine disorder, " lactation tetany " or " grass tetany," has been definitely associated with pronounced hypomagnesæmia (Sjollema 1930, Dryerre 1932, Blakemore and Stewart 1933) with or without concomitant hypocalcæmia (Allcroft and Green 1934). A certain similarity in nervous symptoms between equine " transit tetany " and bovine " grass tetany " suggested that the former might also reveal hypomagnesæmia if looked for, and arrangements were therefore made to watch for cases on the occasion of the October influx of ponies into the Bangor district of North Wales for the Menai Bridge Fair in 1934. Five subacute cases were obtained and the data which follow show the anticipated biochemical feature quite clearly.

History of Cases.—A miscellaneous batch of twelve ponies reared on mountain or moorland pasture in middle Wales had been bought for resale at the Menai Bridge Fair, walked about three miles to a railway station and trucked at 1 p.m. on October 15th. They arrived at Llanfair at 10 a.m. on the 16th and were unloaded at 1 p.m. Of the twelve animals four showed distinct clinical symptoms of " transit tetany " although all were mild cases for which prognosis of spontaneous recovery was good; one was regarded as " doubtful tetany " and the remaining seven were clinically normal. Blood samples were taken from all twelve. The four diagnosable cases made an uneventful recovery but were still available for a second blood sampling on October 30th, by which time the remainder of the truck-load had been dispersed and could not be resampled. In order to compare the

ostensibly normal ponies of the affected truck with genuinely normal equines of random origin blood samples were taken from a miscellaneous selection of horses, mainly in Berkshire.

Data.—Table I gives the serum Ca and serum Mg values for the Welsh ponies and Table II the data for normal horses taken at random. Both are charted in Fig. 1 along with a selection of Weybridge data on bovine cases with which it is desired to institute comparison.

TABLE I.

Serum Ca and Mg. in mgs. per 100 m.l. in trucked Welsh Ponies.

		16*th October*, 1934.		30*th October*, 1934.	
		Ca	*Mg*	*Ca*	*Mg*
A	White mare, affected	5·55	1·49	11·25	2.27
B	Strawberry roan mare, slightly affected ...	6·55	0·96	11·35	2·32
C.	Roan mare, slightly affected	6·70	0·87	11·35	1·82
D.	Light roan mare, slightly affected	7·90	1·33	11·60	2·06
E.	Mare, doubtful diagnosis	9·90	1·51		
F.	Normal mare	11·85	2·00		
G.	Normal mare	10·85	1·94		
H.	Normal mare	11·05	1·73		
I.	Normal yearling filly	11·55	1·91		
J.	Filly foal of A. Aged 5 months	11·45	1·88		
K.	Filly foal of C. Aged 6 weeks	10·75	1·70		
L.	Colt foal of D. Aged 3 months	11·55	1·59		

TABLE II.

Serum Ca and Mg. in mgs. per 100 m.l. in Normal Horses.

	Ca	*Mg*		*Ca*	*Mg.*
Riding hack 1	10·65	1·90	Cob mare 1 ...	12·35	1·92
Riding hack 2	11·95	1·89	Cob mare 2 ...	11·20	2·10
Riding hack 3	11·00	2·21	In-foal mare 3 ...	11·95	2·07
Riding hack 4	12·60	2·20	In-foal mare 4 ...	11·85	1·82
Riding hack 5	12·95	2·12	In-foal mare 5 ...	12·15	2·04
Riding hack 6	10·70	2·06	In-foal mare 6 ...	12·05	2·11
Grey Welsh pony ...	11·65	1·80	In-foal mare 7 ...	12·30	2·00
Draught mare at grass ...	13·70	2·00	In-foal mare 8 ...	12·20	1·88
Draught gelding at grass ...	13·10	1·90	In-foal mare 9 ...	11·85	1·89
			In-foal mare 10 ...	11·30	2·07

Discussion.

It will be observed from Table I that all four cases about which clinical diagnosis was not in doubt show marked hypocalcæmia and equally pronounced hypomagnesæmia, the average Ca figure being 6·67 mg. and average Mg figure being 1·16 mg. per 100 c.c. serum as against 11·14 mg. Ca and 2·12 mg. Mg for the same four animals a fortnight later. Individual values cannot be related to the severity of the symptoms in individual cases, but close correlation could hardly be expected in view of the fact

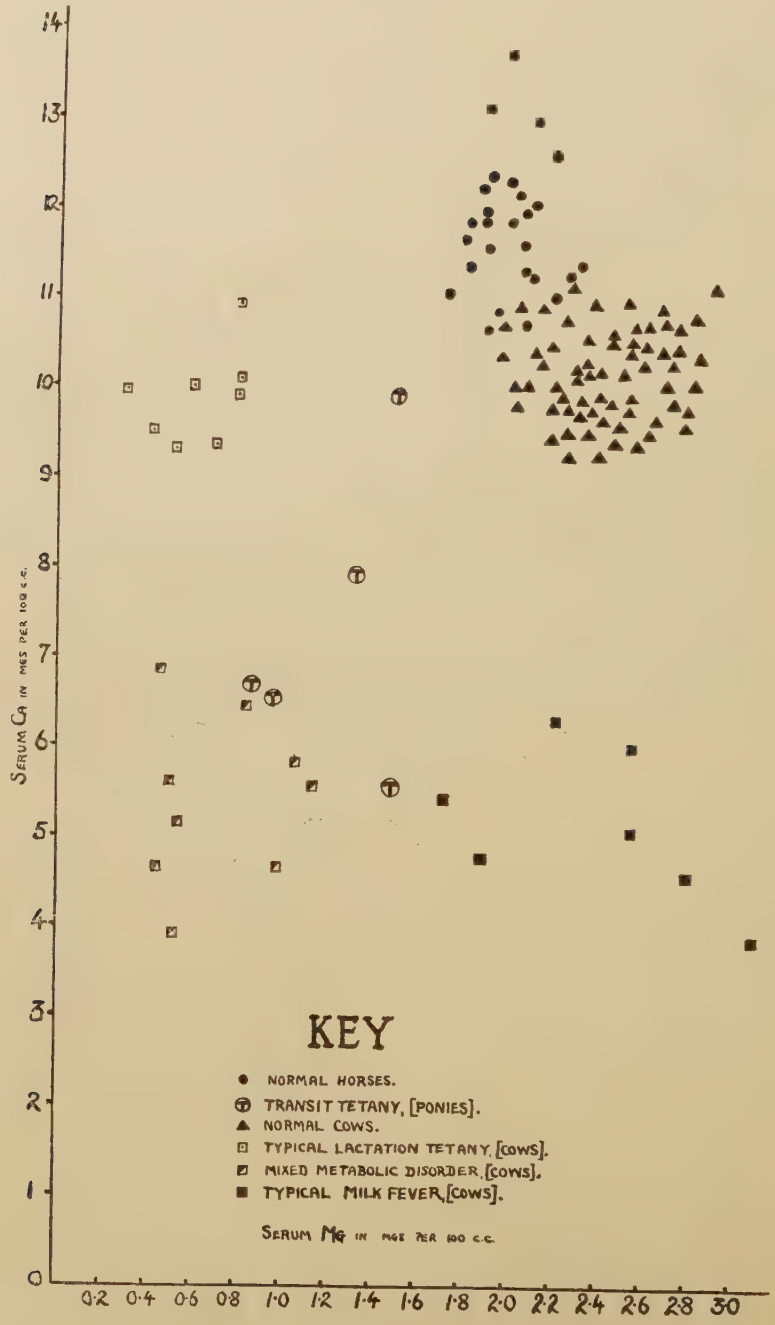

FIG. 1.

Spot chart for values of Calcium and Magnesium in the Serum of Cows and Horses, normal and under pathological conditions.

that all were mild cases, not easy to arrange in order of severity in the temporary environment of a railway siding. The clinically doubtful case, however, shows a calcium value not far removed from normal and a degree of hypomagnesæmia much less marked than the " slightly affected " ponies B and C. A recovery sample was not available for this case, nor could the individual values of the seven " clinically normal " ponies from the same truck be ascertained after a lapse of time sufficient to dissociate the data from the influence of the railway journey. Comparison with Table II, however, does not permit any of them to be treated as definitely abnormal although H, K and L might legitimately be suspected of slight hypomagnesæmia and of hyperexcitability not apparent to the naked eye.

More instructive than the tabulated data is the spot chart presented in Fig. 1. This attempts to correlate the mixed hypocalcæmia and hypomagnesæmia of the equine with a biochemically similar condition in the bovine which came under observation in middle west England earlier in the same year, and to contrast the straight hypocalcæmia of " milk fever " and straight hypomagnesæmia of " lactation tetany " in cows. The comparative data for the bovine are taken from a previous publication (Allcroft and Green, 1934) and the legend of the chart is self-explanatory. The points to which it is desired to draw attention are :—

(1) The values for serum Ca are distinctly higher and for serum Mg distinctly lower in the normal equine than in the normal bovine. Extremes overlap and it would not be possible to differentiate sera on the Ca-Mg figures alone, but the statistical trend of values is obvious.

(2) Hypomagnesæmia is the characteristic feature of typical " lactation tetany " (grass tetany) occurring independently of calving date, characterised by nervous hyperirritability and commonly terminating in a convulsive seizure. Hypocalcæmia, with a suggested trend towards hypermagnesæmia, is the characteristic feature of " milk fever " occurring shortly after calving and commonly terminating in coma. A mixed metabolic disorder occurs in the bovine at any time, in which the symptom complex is often so ambiguous as to render clinical diagnosis very difficult and in which both hypocalcæmia and hypomagnesæmia are pronounced. In general, the symptoms of hypomagnesæmia are the more prominent and any periodic evidences of nervous hyperirritability tend to lead to a diagnosis of tetany.

(3) The " transit tetany " of the Welsh mountain poines tends to simulate " atypical lactation tetany."

In the earlier paper of Montgomerie, Savage and Dodds the hyperpnœa and alkalosis resulting from the conditions under which the ponies had travelled were credited with playing an important part in the genesis of the syndrome, but in view of the observations of McCance (1932) on hyperventilation tetany in the

human subject, in which neither hypomagnesæmia nor hypocalcæmia was shown, the matter needs more detailed experimental consideration.

Of particular interest in comparing the equine disorder with the corresponding bovine disorder is the fact that similar syndromes appear under converse environmental changes. In England the incidence of " lactation tetany " is so seasonal that as many cases occur in April or May as in all the other months of the year put together. In Holland the association between the incidence of the disease and the turning of the cows out to pasture is so definite that the name " grass tetany " is used to describe the same disorder, and considerable energy has been expended in searching for some positive factor (nitrate, nitrite, K/Na ratio, protein aporrhegmas, sensitising factors) which might account for the metabolic disturbance. In the case of our Welsh mountain ponies the outbreak of a hypomagnesæmic, hypocalcæmic, symptomatically similar disorder occurred as the result of the reverse transfer from freedom at grass to confinement and fear; it may occur within 21 hours on a railway journey in horses of both sexes and on simple transfer from pasture to stall in the case of mares with foal at foot. The suggestion is that the disturbance is primarily cortical.

In both species of animal the disorder is uncommon in the male but at least two cases have been recorded in stallions and two in bulls. It has also been recorded in a barren cow and in a barren mare, but by far the greatest incidence is in lactating cows and in suckling mares.

It is suggested that there is a group of clinically separable disorders involving disturbance of Ca and Mg levels in the blood, for which a common physiological explanation will ultimately be found, but little purpose would be served by speculating further until more data are acquired concerning the physiological mechanisms for controlling serum Mg and serum Ca. Meanwhile our observations on the equine seem worthy of immediate record.

Summary.

Pronounced hypomagnesæmia, as well as hypocalcæmia, is shown to be a characteristic feature of " transit tetany " occurring in pasture-reared horses, generally suckling mares, during or after the disturbance of a long railway journey, or of the transfer from free range to confinement. The condition is compared with "grass tetany" of the bovine occurring most commonly after transfer of cows from winter stall conditions to spring pasture, although not restricted to such environmental change and occasionally occurring in breeds at grass all the year round. The symptom complex and biochemical blood picture in the equine is more analogous to atypical " grass tetany " in the bovine than to the typical disorder characterised by comparatively straight hypomagnesæmia,

and is easily distinguished from " milk fever " characterised by hypocalcæmia with normal or even slightly elevated serum magnesium.

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REVIEW.

Die Unsichtbaren Krankheitserreger Filtrierbare Vira. Ein Lehrbuch für Ärzte und Tierärzte. Von Dr. Josef Baló, o.ö Professor de pathologischen Anatomie und Pathohistologie an der Franz-Joseph, Universität Szeged. Berlin, 1935, S. Karger. Price R.M. 24.

The text of this work extends to 301 royal 8vo pages. The first 40 pages deal in a general way with the filterable viruses, including such subjects as their size, cultivation, staining, affinity for particular tissues, cell inclusions, immunity, etc. The remainder, divided into 10 chapters, is devoted to an account of all the diseases of man and the domesticated animals which can be said to have established their claim to be regarded as caused by such viruses. These are arranged in six groups : (1) Pox diseases of man and animals ; (2) Herpes and similar diseases ; (3) Neurotropic filterable viruses ; (4) Organotropic viruses ; (5) Virus diseases of a general septicæmic type caused by filter passing viruses ; (6) Bryocytoses, tumours caused by filterable viruses.

Without flattery it may be said that the work is one of exceptional merit, not only on account of the fullness of the information which it provides regarding the filterable viruses, but also because of the clear, logical way in which the facts are presented, and the evidence examined where opinions are not unanimous. The information is well up to date.

There are 39 good figures in the text, mostly illustrating the histology of different diseases caused by filterable viruses, and three coloured plates.

The author's style makes the book very easy to read, and the print and paper are good.

ABSTRACT

THE PATHOGENESIS AND EXCRETION OF THE BACILLI IN BOVINE PARATUBERCULOSIS (JOHNE'S DISEASE).

By Dr. N. A. Alexejeff-Goloff.

Apart from examination of Johne's disease in experimental animals (guinea-pigs, rabbits, pigeons, fowls) in which paratuberculosis occurs only as a bacteriæmia resulting from experimental infection, the author states that he has submitted to careful bacteriological examination the organs and tissues of four cows that were affected with the disease. During life these animals showed the pronounced clinical symptoms of Johne's disease (persistent diarrhœa in spite of treatment, and great emaciation). During the bacteriological examination of the fæces and scrapings from the mucous membrane of the rectum acid-fast, alcohol-fast and antiformin-resistant clusters of Johne bacilli were recognised in all four animals. One of the cows died from the disease and the three others were killed in a slaughter house. In all the animals there was great enlargement of the mesenteric lymphatic glands and different degrees of pronounced paratuberculosis changes in the small and large intestine. In one of the cows necrotic centres were present in the liver and the mucous membrane of the bile and urinary bladders was thickened. Pure cultures of the bacilli were obtained from all four animals.

In three of the cows the milk was examined during life. Strict precautions against accidental contamination were observed in taking the samples of milk, and in all the specimens examined acid-fast and antiformin-fast heaps of Johne bacilli were detected, and cultures were obtained from the specimens withdrawn with a milk catheter from the udder.

During a period of 15 days milk from one of the cows was fed to a three months' old puppy, to which previously 2 c.c. of ox gall had been given to sensitise and diminish the resistance of the intestine. At the end of 15 days the puppy had become notably reduced in condition, and it was then killed. The post-mortem showed catarrh of the duodenum and jejunum, and also enlargement of the related mesenteric lymphatic glands. In the bacteriological examination acid-fast and antiformin-fast paratuberculosis bacilli were found in the mucous membrane and in the enlarged lymphatic glands. Cultures were obtained from the diseased parts of the intestine.

On post-mortem bacteriological examination of the lymphatic glands and tissue of the udder of the cows that were killed, characteristic Johne bacilli were found in all the cases.

At the post-mortem examination of one of the slaughtered cows urine was taken from the bladder and Johne bacilli were recognised in it. In bacteriological examination of the blood of the heart and of the peripheral vessels, lymphatic glands, heart muscle, lungs, liver, spleen, suprarenals, kidneys, gall bladder, urinary bladder, ovaries, mesenteric lymphatic glands, and mucous membrane of the intestine, in all four cows there were found antiformin-resistant and acid-fast clusters of bacilli. At the same time there were found acid-fast and antiformin-fast small granular rods. Pure cultures of the bacilli were obtained from the organs.

At the slaughter of one of the cows during the third month of pregnancy examination of the uterus showed clusters of acid-fast and antiformin-fast bacilli which could be recognised to be Johne bacilli in the fœtal envelopes

and fluids, and in the blood, liver, and in all the organs and tissues. A pure culture of the bacilli was obtained from the fœtus.

One cow (No. 20) which began to show symptoms three weeks before parturition gave birth to a normal calf on 17/6/29. Immediately after the birth the calf was completely isolated from its dam and fed with the milk of a healthy cow from the dairy. On the 10/7/29 the calf was submitted by the author to a careful bacteriological examination. On examination of the fæces and of urine taken with a catheter, and of scrapings from the mucous membrane of the vagina and intestine, and of the peripheral blood, acid-fast and antiformin-fast characteristic Johne bacilli were found. Similar examinations repeated after two or three days had the same result. Throughout the whole of the time the calf appeared quite well, developed normally, had a normal temperature and showed no signs of Johne's disease.

On 18/7/29 the calf was submitted to protective inoculation against anthrax by the combined method of serum and vaccine subcutaneously. Six hours after the inoculation the calf showed a febrile temperature and strong diarrhœa set in, but after some hours recovery took place. Bacteriological examination of the fæces and peripheral blood now showed great numbers of characteristic Johne bacilli. The protective inoculation in this case thus had the effect of bringing to light the disease which previously had been running an unobserved course.

On the basis of these facts, in the bacteriological examination made during life for the purpose of diagnosis there should be examined, in addition to the fæces and scrapings from the mucous membrane of the rectum, the blood, urine and milk when Johne's disease is suspected.

Conclusions.—Johne's disease in the ox runs in the form of a bacteriæmia in the course of which in pregnant animals intra-uterine infection of the fœtus occurs.* This fact must be taken into account in carrying out prophylactic and hygienic measures against Johne's disease. (*Zeits. für Infekt. Krank. der Haus*, 1929, vol. xxxvi, pp. 313-317.)

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*In the opinion of the Editor, this conclusion should meanwhile be treated with incredulity and regarded as the result of errors in observation.

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